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When is a "cryptic" species not a cryptic species: A consideration from the Holarctic micro-landsnail genus *Euconulus* (Gastropoda: Stylommatophora)

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ABSTRACT

Naive use of molecular data may lead to ambiguous conclusions, especially within the context of "cryptic" species. Here, we integrated molecular and morphometric data to evaluate phylogenetic relationships in the widespread terrestrial micro-snail genus, *Euconulus*. We analyzed mitochondrial (16S + COII) and nuclear (ITS1 + ITS2) sequence across 94 populations from Europe, Asia and North America within the nominate species *E. alderi*, *E. fulvus* and *E. polygyratus*, and used the southeastern USA *E. chersinus*, *E. dentatus*, and *E. trochulus* as comparative outgroups. Phylogeny was reconstructed using four different reconstruction methods to identify robust, well-supported topological features. We then performed discriminant analysis on shell measurements between these genetically-identified species-level clades. These analyses provided evidence for a biologically valid North American "cryptic" species within *E. alderi*. However, while highly supported polyphyletic structure was also observed within *E. fulvus*, disagreement in placement of individuals between mtDNA and nDNA clades, lack of morphological differences, and presence of potential hybrids imply that these lineages do not rise to the threshold as biologically valid cryptic species, and rather appear to simply represent a complex of geographically structured populations within a single species. These results caution that entering into a cryptic species hypothesis should not be undertaken lightly, and should be optimally supported along multiple lines of evidence. Generally, *post-hoc* analyses of macro-scale features should be conducted to attempt identification of previously ignored diagnostic traits. If such traits cannot be found, i.e. in the case of potentially "fully cryptic" species, additional criteria should be met to propound a cryptic species hypothesis, including the agreement in tree topology among both mtDNA and nDNA, and little (or no) evidence of hybridization based on a critical analysis of sequence chromatograms. Even when the above conditions are satisfied, it only implies that the cryptic species hypothesis is plausible, but should optimally be subjected to further careful examination.

1. Introduction

Molecular methods have become one of the most powerful tools to empirically evaluate taxonomic hypotheses (e.g. Bickford et al., 2007; Hillis, 1987; Sáez and Lozano, 2005). The novel insights gained from these approaches often lead to the re-evaluation of formerly accepted taxonomic entities (Beheregaray and Caccone, 2007; Knowlton, 2000; Trontelj et al., 2009), and significantly impact the goals and optimum management of biodiversity conservation (Agapow et al., 2004; Soltis and Gitzendanner, 1999). In spite of its analytical power, however, naive use of molecular data is not without serious taxonomic issues (Bickford et al., 2007). For instance, the cut-off boundaries between phylogenetic groups are ultimately subjective and typically based on qualitative opinion (Agapow et al., 2004; Horvath, 1997). And, because reconstructed phylogenetic pattern is often a function of sampling intensity and biogeographic coverage (Heath et al., 2008), it is also difficult to know if a given entity would be robust across multiple sample sizes and scales.

Molecular analyses have variously documented under- and over-reporting of biological diversity. The former can occur when organisms classified to a single species with apparently similar macro-scale traits are shown to reside in multiple highly-supported monophyletic genetic clades. Commonly referred to as "cryptic species", these entities are often left without taxonomic descriptions (e.g. Bickford et al., 2007; Tan et al., 2010). Obviously, not always is it possible or appropriate to provide a formal description of a new species as soon as it is delineated (Dayrat, 2005); the problem stems from the fact that for many cryptic species a formal description is *never* attempted (Schlick-Steiner et al., 2007). Moreover, a consensus is still lacking about how we define "cryptic" (e.g. de León and Nadler, 2010; Struck et al., 2018), or

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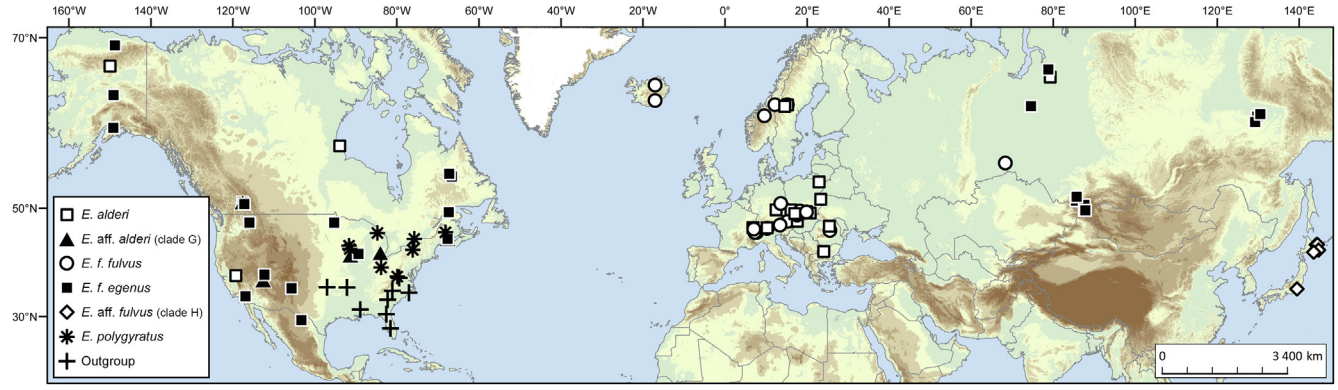

Fig. 1. Geographic distribution of sites at which the analyzed *Euconulus* specimens were collected. For phylogenetic relationships among taxa see Fig. 2.

whether these species even truly exist as a natural phenomenon, or just as a temporary taxonomic problem (Heethoff, 2018). Essentially, there are two approaches towards this issue. Bickford et al. (2007) in their highly influential work refer to cryptic species as to those that have been classified under one species name due to at least superficial morphological similarity. From this perspective, cryptic species merely result from erroneous taxonomic conclusions, while additional *post-hoc* research often leads to the detection of formerly overlooked morphological characters (e.g. Knowlton, 2000; Schlick-Steiner et al., 2007; Tan et al., 2010). Others argue that "fully cryptic" species are only those for which differences in morphology cannot be detected (Jörger and Schrödl, 2013). A possible later identification of morphological characters means that they are no longer cryptic, being referred to as "pseudo-cryptic" species (Sáez and Lozano, 2005).

As opposed to the cryptic species discoveries, molecular research can also document unwarranted exuberance on the part of taxonomists. This has been frequently documented in marine (e.g. Knowlton, 2000; Raith et al., 2016; Wray et al., 1995) and non-marine molluscs (e.g. Köhler and Burghardt, 2016; Simison and Lindberg, 1999; Teshima et al., 2003; Nekola et al., 2015, 2018) where high levels of intraspecific shell trait plasticity exist (e.g. Emberton, 1995; Haase and Misof, 2009; Köhler and Burghardt, 2016; Nekola et al., 2015, 2018). In these situations various shell forms within a given species-level genetic clade have each been assigned a separate species-level *nomen*. The integration of genetic with traditional data streams is therefore often considered vital to ensure that the species-level hypotheses are reliable and well-vetted (e.g. Bickford et al., 2007; Köhler and Johnson, 2012; Tan et al., 2010). To not do so could lead to the generation of spurious taxonomic concepts which would inflate biodiversity estimates and negatively impact conservation planning and ecological research (e.g. Bickford et al., 2007; Trontelj and Fišer, 2009).

Most documented over-splitting in molluscs has been reported in large taxa (Agapow et al., 2004; Knowlton, 2000; Trontelj and Fišer, 2009). It is not known to which extent this pattern can be extrapolated to micro-snails (< 5 mm), however, two current revisionary works on genera *Pupilla* (Nekola et al., 2015) and *Vertigo* (Nekola et al., 2018) show that both over-splitting and over-lumping have taken place. These species are unique because of their often large (1000 + km maximum extent) ranges (Nekola et al., 2009), which are at least partially a function of their highly effective passive dispersal abilities (Rees, 1965; Wada et al., 2012) in combination with their greater incidence of uniparental reproduction (e.g. Pokryszko and Cameron, 2005). In spite of this, only a few micro-snail phylogenetic studies have been based on continental to global-scale data sets (Nekola et al., 2009, 2015, 2018; Weigand et al., 2013). As a result, most published works (e.g. Schilthuizen et al., 2005; Tongkerd et al., 2004; Wada et al., 2013) are not capable of documenting range-wide evolutionary pattern and process. As micro-snails represent a substantial proportion of global terrestrial gastropod diversity (Welter-Schultes, 2012), especially at small observational scales (Myšák et al., 2013; Nekola, 2014), re-evaluation of their morphology-based taxonomy is crucial for correct biodiversity estimates and conservation concerns.

Recent Holarctic micro-snail work (Nekola et al., 2015, 2018) has documented allopatric replacement amongst multiple groups of "cryptic" species, which were also shown via *post-hoc* analyses to possess unique shell features. Inspired by these results, we decided to examine the terrestrial micro-gastropod *Euconulus* Reinhardt, 1883. Members of this genus are common throughout temperate and boreal Eurasia and North America. While some taxa are endemic to southeastern North America (Hubricht, 1985; Pilsbry, 1946), *E. fulvus* (O. F. Müller, 1774) and *E. alderi* (Gray, 1840) are reported from both continents (Welter-Schultes, 2012; Nekola, 2014). Because of wholesale taxonomic confusion regarding species diversity and nomenclature within the genus (Welter-Schultes, 2012) – largely due to their very simple and plastic conchology – a phylogenetic analysis of *Euconulus* across its global extent is of interest. We set two major aims for this study:

(1) To empirically document global taxonomic diversity within the genus *Euconulus*, focusing in particular on the Holarctic *E. fulvus* and *E. alderi*, using a consensus across mtDNA sequence, nDNA sequence, shell morphometrics, and ecology/biogeography;
(2) To explore the potential presence of cryptic species within the genus, and propose basic criteria that need to be met to propound a cryptic species hypothesis.

2. Material and methods

2.1. Data collection

A total of 94 individuals were sampled from the Holarctic, extending from Iceland and across Eurasia to encompass all of North America (Fig. 1). Detailed site descriptions are available in Appendix A. *A priori* species assignments were based on currently recognized diagnostic conchological and body features as reported by Horsák et al. (2013), Kerney and Cameron (1979), Nekola (2003) and Pilsbry (1946). Specimens assigned to *E. fulvus* had pale bodies, squat conical shells of pale yellowish-brown color, silky upper surface and very faint or absent spiral lines on the ventral surface. While sometimes straying into wetland habitats, this species is principally limited to mesic uplands. We use *E. f. fulvus* to designate European material and *E. f. egenus* (Say, 1825) – the earliest *nomen* for non-European material – to designate Central Asian to North American populations. Specimens assigned to *E. alderi* had dark bodies with darker shells of glossy lustre and distinct spiral lines on the ventral surface. As recommended by Welter-Schultes (2012), we use *E. alderi* instead of *E. praticola* (Reinhardt, 1883). This species is strictly limited to wetland sites. *Euconulus chersinus* (Say, 1821), *E. dentatus* (Sterki, 1893), *E. trochulus* (Reinhardt, 1883) and *E.*

Table 1
Forward (F) and reverse (R) primer sequences used for genetic analysis, anneal temperatures for PCR, and authors of primer design.

Region	Sequence	Anneal	Source
COII (F)	5′-AAATAATGCTATTTCATGAYCAYGC-3′	45 °C	Hugall et al. (2002)
COII (R)	5′-GCTCCGCAAATCTCTGARCAYTG-3′	45 °C	Hugall et al. (2002)
16S (F)	5′-GCGCTGTTTATCAAAAACAT-3′	52 °C	Palumbi et al. (2002)
16S (R)	5′-CCGGTYTGAACTCAGATCAYGT-3′	52 °C	Palumbi et al. (2002)
ITS1 (F)	5′-TAACAAGGTTTCCGTATGTGAA-3′	52 °C	Armbruster and Bernhard (2000)
ITS1 (R)	5′-TCACATTAATTCTCGCAGCTAG-3′	52 °C	Author design
ITS2 (F)	5′-CTAGCTGCGAGAATTAATGTGA-3′	52 °C	Wade and Mordan (2000)
ITS2 (R)	5′-GGTTTCACGTACTCTTGAAC-3′	52 °C	Author design

polygyratus (Pilsbry, 1899) all possess shells with tighter coiling than the *fulvus/alderi* group. These eastern North America taxa have been used for outgroup comparisons – and not taxa from outside of the genus – due to difficulties with between-genus alignment of ITS1 and ITS2 nDNA amplicons (Nekola et al., 2009, 2018).

2.2. DNA extraction, PCA amplification and sequence analysis

Specimens were either preserved in absolute ethanol, or allowed to desiccate at ambient temperature and humidity. DNA was extracted using the E.Z.N.A. Mollusc DNA Kit (Omega BioTek) and stored at −20 °C. Due to the poor diffusion ability of proteinase into and liberated DNA out of these small, tightly coiled shells, shell destruction was necessary for sufficient DNA yield. All specimens were microscopically imaged prior to shell destruction using standard methods (Nekola et al., 2009, 2018). Shell images are available upon request.

Amplicons for two mitochondrial genes [16S ribosomal RNA (16S) and cytochrome oxidase subunit II (COII)], and two nuclear genes [ribosomal internal transcribed spacers ITS1 and ITS2], were generated using primers and protocols as listed in Table 1. PCR products were purified using ExoSAP (Affymetrix) and cycle sequenced in forward and reverse directions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Forward and reverse strands were assembled into one sequence using Geneious v. 8.0.2 (Biomatter Ltd.) and checked by eye for potential misreads. IUPAC ambiguity code was used to represent heterozygous positions in nDNA sequences, i.e. in the cases when two bases at a given position in the chromatogram expressed the same peak height. For the less variable ITS1 + ITS2 constructs, base pair variation is illustrated in matrix form, showing the makeup of heterogeneous sites and the location of all insertions/deletions. All sequences were deposited in GenBank (Appendix A).

2.3. Phylogenetic analysis

Sequences were aligned using ClustalX, using the default parameters of the IUB weight matrix as implemented in MEGA v. 6.0 (Tamura et al., 2013), and checked by eye for potential errors. Phylogenetic analysis was conducted separately using concatenated mitochondrial 16S + COII and nuclear ITS1 + ITS2 fragments. We used four different methods of phylogeny reconstruction – each based on very different analytical assumptions – to robustly identify well-supported topological features. MEGA v. 6.0 was used to infer phylogenetic trees by Neighbor-joining based on maximum composite distance including transitions and transversions with pairwise gap deletion. Maximum Parsimony analysis was conducted in TNT (Goloboff et al., 2008) using the traditional search option with 1000 replicates of Wagner trees, one random seed, tree bisection reconnection branch swapping algorithm and 10 trees to save per replication. The datasets were partitioned by genes, and by creating two separate partitions for the protein coding COII, one for the combined 1st + 2nd codon positions, and one for the 3rd codon positions. The best-fitting models for each partition were chosen using jModelTest v. 2.1.10 (Darriba et al., 2012; Guindon and Gascuel, 2003) based on the Bayesian Information Criterion. Using the selected models, Bayesian trees were constructed in MrBayes v3.2.6 (Huelsenbeck and Ronquist, 2001), simultaneously running one cold and three heated MCMC chains for 10 000 000 generations with a sample frequency every 1000 generations. The first 25% of trees were discarded as burn-in, while the remaining samples were used to construct a consensus tree and calculate Bayesian posterior probabilities. Four independent searches were run and checked for consistency in Tracer 1.6 (Drummond and Rambaut, 2007). The searches were considered stable and convergent when effective sample sizes of all parameters exceeded 200 and standard deviation of split frequencies fell below 0.01. Maximum Likelihood analysis was performed in RAxML v 8.2 (Stamatakis, 2014) with 500 search replicates, using the GTRGAMMA models for separate gene partitions within the concatenated mtDNA and nDNA datasets. Node support was assessed with 1000 nonparametric bootstrap replicates (Felsenstein, 1985). For the tree topologies obtained, bootstrap support values above 70% for NJ, MP and ML, and Bayesian posterior probabilities above 95% were considered significantly supported and shown in the phylogenetic trees. Trees were visualized using FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

2.4. Post-hoc species delimitations

Provisional species-level clades based on DNA sequence data were reciprocally-monophyletic in both nuclear and mitochondrial DNA phylogenetic trees, and possessed high support values in mtDNA. Support values in nDNA were of limited applicability because of the small number of variable positions (~60 informative sites across ~1500 bp). We therefore created a base pair and insertion-deletion matrix and inspected it by eye to identify potential diagnostic differences between clades. A similar character-based approach has been proven to help species delimitations by identifying unique bases or strings of diagnostic bases in molecular taxonomy (e.g. Nekola et al., 2015; Rach et al., 2008; Zielske and Haase, 2015). Cases of topological incongruence between mtDNA and nDNA trees were identified, with conchology being used to designate which sequence was expected for that specimen. Putative species-level clades were subsequently subjected to conchological analysis (see below), and visual evaluation of qualitative morphological features. Initial (*a priori*) species level identifications were then adjusted to correspond to this integrative analysis.

2.5. Shell morphometrics

Shells selected for analysis encompassed the range of observed variability among those remaining in a given lot following removal of some for DNA extraction. On average three shells were selected per lot. Chosen lots/populations covered as wide a geographic and ecological range as possible. The normality of shell measures was checked by histograms and by the Shapiro-Wilk (W statistic) normality test. The analyzed dataset compromises: European/Beringian *Euconulus alderi*, N = 27 [seven populations from Czech Republic, Slovakia, Poland, Bulgaria, Sweden, Russia (Western Siberia) and USA (Alaska)]; North

American *E.* aff. *alderi*, N = 14, [four populations from USA (Iowa, Maine and Utah) and Canada (British Columbia)]; *E. f. fulvus*, N = 16 [six populations from Czech Republic, Norway, Switzerland and Russia (Western Siberia)]; *E. f. egenus*, N = 35, [twelve populations from Russia (Altai and Western Siberia), Canada (British Columbia and Québec), and USA (Alaska, California, Texas, and Utah)]; *E. polygyratus*, N = 14, [four populations from USA (New York, Ohio and Virginia), and Canada (Ontario)]. Japanese *E. fulvus* material was excluded due to lack of fully mature individuals for measurement.

Shell width and height, aperture width and height, body whorl height, and number of whorls were measured via microscope images of each shell from top and frontal views, using an Olympus SZX7 with Olympus C-7070 Wide Zoom camera and QuickPHOTO MICRO image analysis software. Five to seven sequential, stacked in-focus images were taken from the bottom to top of each shell with a single, focused composite image being generated via Deep Focus software.

Because it is impossible to know with certainty when shell growth has ceased in *Euconulus* (e.g. by the formation of apertural structures), we limited analysis to only shells of more than four whorls. We used ratios between measured shell characteristics, i.e. shell width/number of whorls, shell height/number of whorls, shell height/shell width, body whorl height/shell height, aperture width/shell width, aperture width/shell height, and aperture height/body whorl height. Discriminant analysis was performed on these data, with measured characters being linearly fitted into a two-dimensional ordination space. Identification of the important characters for taxa delimitation was tested by 4999 random permutations. All calculations were done in R version 3.3.1 (R Core Team, 2016), using the “ade4” (Dray and Dufour, 2007) and “vegan” (Oksanen et al., 2017) packages.

These measurements were complemented by visual evaluation for additional qualitative morphological features, including shell shape from the apertural view (flattened vs. conical, presence of keel), tightness of coiling from the apical view, surface color and structure (lustre, microstructure, presence of ribs), presence of bottom spiral grooves, animal tissue color and mantle color, and protoconch and teleoconch microsculptures. The latter two features were viewed using the digital microscope Keyence VHX-5000 with ZS-20 and ZS-200 objective lenses.

Genital anatomy was not evaluated, given that the majority of samples were preserved as mummified specimens, making the tissue dissections impossible. Additionally, genital structures of *Euconulus* are rather simple (Pilsbry, 1946), making it unlikely that reliable species-specific features exist. Genital anatomy has proven to be of little taxonomic use in other micro-snails such as *Pupilla* (Pokryszko et al., 2009).

3. Results

Sequence data were obtained for 91 specimens for 16S + COII fragment, and 93 specimens for ITS1 + ITS2 fragment (Appendix A). The amplicons of 16S, COII, ITS1 and ITS2 consisted of 378–381, 502, 652–664 and 862–890 bp, respectively. Both mtDNA and nDNA fragments could be unambiguously aligned. The 16S, COII, ITS1 and ITS2 amplicons contained 36, 134, 33 and 28 variable sites, respectively. The total length of concatenated fragments was 881–883 bp for 16S + COII, and 1514–1553 bp for ITS1 + ITS2.

3.1. Phylogenetic analyses and supported clades

Phylogenetic reconstructions possessed essentially identical tree topologies across the four different methods (i.e. NJ, MP, ML, and BI) in both datasets. Therefore, only one representative tree (ML) is shown for mtDNA and nDNA (Fig. 2). Node support values were in general lower for nDNA vs. mtDNA data, presumably due to the much lower number of variable base pairs in the former. Additionally, support values differed among the reconstruction methods, with BI posterior probabilities giving conspicuously higher support than the three other methods especially in the nDNA tree (Fig. 2).

Both mtDNA and nDNA revealed that *E. polygyratus* (clade F) is a member of the same highly supported clade that contains all other *E. fulvus/alderi* group members. As a result it was relocated to the target species set.

While nDNA clade support values were lower than for mtDNA, the variable site matrix (Table 2) consistently demonstrated diagnostic base pairs and/or insertions-deletions for each major clade. Clade A (*E. alderi*) was distinguished by 249 T and 537A in the concatenated ITS1 + ITS2 construct. Clade B (European *E. f. fulvus*) was largely distinguished by 572A, 830G, 956C and 957A. Clade C (North American *E. f. egenus*) was largely distinguished by 559C and a T insert at 424. Clade D (Beringian *E. f. egenus*) was largely demarcated by 604G and a GA insert starting at 342. Clade E (central Asian *E. f. egenus*) was distinguished by 1096T and 1112A. Clade F (northeastern North American *E. polygyratus*) was distinguished by 917C. Clade G (North American *E.* aff. *alderi*) was distinguished by 814A, and 1019T. Clade H (Japanese *E. fulvus*) was largely distinguished by 76A, 87A, and 225T. In addition, association between clades A and B is suggested by 197T and 1012C.

Comparisons between mtDNA and nDNA document that the *E. fulvus/alderi* group globally consists of at least four reciprocally monophyletic clades, which were all highly-supported by mtDNA (for nDNA the support values were lower and a few individuals could not be unambiguously assigned to clades, e.g. E63 and E128, for the reasons described above). The four aforementioned clades included: Eurasian/Beringian *E. alderi* (clade A), European *E. f. fulvus* (clade B), North American *E.* aff. *alderi* (clade G), and North American *E. polygyratus* (clade F). Based on these results we putatively erect clade G to the status of an undescribed new species. However, *E. f. egenus* appears polyphyletic, representing four potentially differentiated clades, at least in nDNA: North American (clade C), Beringian (clade D), central Asian (clade E), and Japanese (clade H). Japanese *E. fulvus* (clade H) appears to be most divergent of all clades in the *E. fulvus* group and may therefore represent a separate entity (at a species or a sub-species level; therefore we hereafter label it as *E.* aff. *fulvus*) but a lack of mature individuals to conduct measurements precluded us to verify this idea. Deeper nodes in mtDNA and nDNA could not be reliably resolved due to low support values in both methods.

3.2. Incongruence between mtDNA and nDNA trees

While the variable base pair/insertion-deletion matrix (Table 2) corroborated the highly supported mtDNA clades and also identified apparent polyphyleticism in *E. f. egenus*, the placement of some specimens was incongruous between mtDNA and nDNA (represented in bold-font in Fig. 2). Among these were two *E. f. fulvus* specimens (E80 and E101) that could not be assigned to any of the major clades within the target group based on their nDNA. The nDNA base variability matrix, however, illustrated that the sequence for these specimens was heterozygous at several base positions, in each case with one base being characteristic of *E. f. fulvus* (clade B) and one of *E. f. egenus* (clade E). Additionally, almost 25% of *E. f. egenus* individuals demonstrated incongruence in their assignment between nDNA and mtDNA clades, with clades C and D lacking support and being substantially mixed in the mtDNA dataset. Lastly, two *E. polygyratus* with nDNA and shells typical of that species were found to constitute a strongly supported subclade rooted within *E. f. egenus* in mtDNA (Fig. 2).

3.3. Quantitative and qualitative conchological variation

Discriminant analysis strongly separated *E. polygyratus* from all other clades (Fig. 3A), with the main gradient of morphological variation being associated with the ratio between the shell width and number of whorls (i.e. coiling tightness; Table 3). After omitting this species and repeating analysis, the remaining entities were more evenly

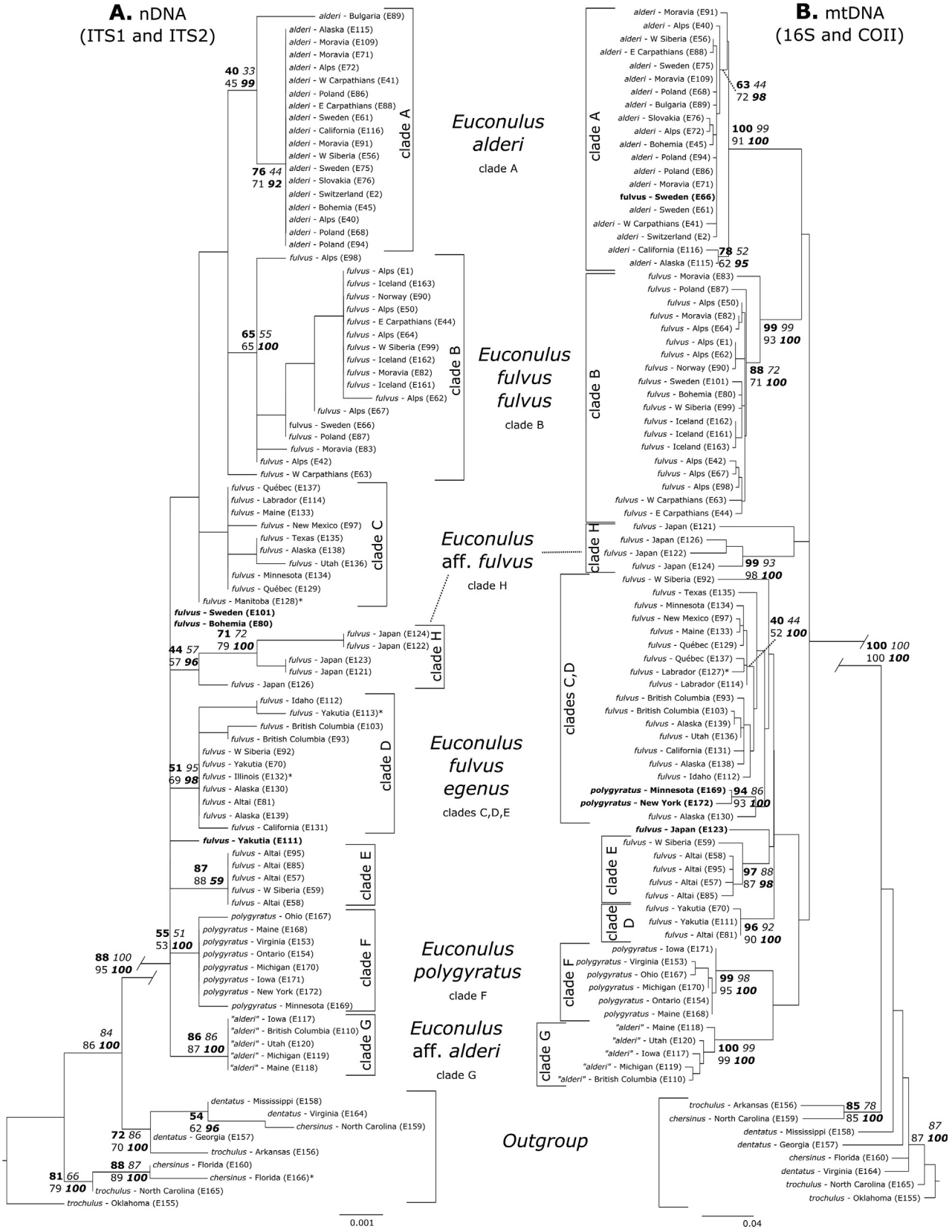

Fig. 2. Maximum likelihood phylogenetic tree reconstruction for *Euconulus* based on concatenated ITS1 and ITS2 fragments of nuclear DNA (A), and 16S and COII fragments of mitochondrial DNA (B). Nodes with high support in at least one of the phylogenetic reconstruction methods (> 70% bootstrap support in NJ, MP, ML and > 95% posterior probability in BI) are labeled with four numbers as follows: NJ, upper left (**bold font**); MP, upper right (*italic font*), ML, lower left (normal font); BI, lower right (***bold italic font***). Species labels at branch tips represent the *a priori* species identifications. Names of putative species and species-level clades, as inferred based on integrative analysis, are placed between the trees next to the corresponding clades. Specimens placed within different clades in nDNA and mtDNA trees are shown in bold in the respective trees. Specimens for which sequence data were not available for either mtDNA or nDNA are marked with an asterisk.

Table 2
Matrix of all variable base positions for ITS1 and ITS2 fragments of nuclear DNA. For heterozygous positions, both bases are shown, separated by slash. Numbers above the matrix refer to the base pair numbers in the concatenated ITS1 + ITS2 fragment downstream of the forward primer. Positions that differ from the genus consensus are highlighted, dashes indicate base-pair deletions. Specimens are grouped together according to the integrative molecular and morphometry analyses. For locality information see the specimen codes in Appendix A.

	ITS1										ITS2										
	1778 5675	8 7	11111 904789 985957	22 24 59	233 823 110	333334444 344572555 223274245	4 7 5	444 999 127	55 03 47	55555 44577 13902	555555556666667 889999990000065 454567890124949	77888 69111 28348	888 366 047	999999999999 113445555555 799890123456	9 5 7	99999999999 55666666666 89012345678	9 6 9	11 999999900 777777811 012345902	11 00 19 96	11 11 01 72	111 111 378 507
***Euconulus alderi* (clade A)**																					
Alps (E40)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alps (E72)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Bulgaria (E89)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-A	CTTGG	CA---------AGGC	CTACG	CCG	TTTCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Bohemia (E45)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-A/C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
E Carpathians (E88)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Moravia (E71)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Moravia (E91)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Moravia (E109)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Poland (E68)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Poland (E86)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Poland (E94)	GCAT/C	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Slovakia (E76)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Sweden (E75)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Sweden (E61)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Switzerland (E2)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
W Carpathians (E41)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
W Siberia (E56)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alaska (E115)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
California (E116)	GCAC	C	CGC-CT	CT	CGA	A--GC-G-C	C	TCT	-A	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
***Euconulus fulvus fulvus* (clade B)**																					
Alps (E1)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC/T	T/A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alps (E50)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA/G	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alps (E62)	GCAC	C	CGC-CT	CC	CTA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alps (E64)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACC/G	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGT/C
Alps (E67)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCG/A	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alps (E42)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCG	CA---------AGGC	CTACG	GCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Alps (E98)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCG	CA---------AGGC	CTACG	GCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	AG	GC	CGC
Bohemia (E80)	GCAC	C	CGC-CC/T	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTC/T	A/T	CCTATGAGGTG	A	GTGAGGCCC/T	CT/G	GA/C	CGC
E Carpathians (E44)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA/G	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Iceland (E161)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Iceland (E162)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Iceland (E163)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Moravia (E82)	GCAC	C	CGC-CT/C	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Moravia (E83)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	--T	-T	CTTCG	CA---------AGGC	CTACG	GCG	TTC---------	-	-----------	-	------CCC	CG	AC	CGC
Norway (E90)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA/G	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC/T
Norway (E66)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
Poland (E87)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA/G	CA---------AGGC	CTACG	GCG	TTCCTATCACTC/T	A/T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGT/C
Sweden (E101)	GCAC	C	CGC-CC/T	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCG/A	CA---------AGGC	CTACG	CCG	TTCCTATCACTC/T	T/A	CCTATGAGGTG	A	GTGAGGCCC/T	CG/T	GA/C	CGC
W Carpathians (E63)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTAG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
W Siberia (E99)	GCAC	C	CGC-CT	CC	CGA	A--GC-G-C	C	TCT	-T	CTTCA	CA---------AGGC	CTACG	GCG	TTCCTATCACTC	A	CCTATGAGGTG	A	GTGAGGCCC	CG	GC	CGC
***Euconulus fulvus egena* (clade C)**																					
Alaska (E138)	GCAC	C	CGC-CC	CC	CGA	A--GCTG-C	C/A	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Labrador (E114)	GCAC	C	CGC-CT	CC	CGA	A--GCTG-C	C	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Maine (E133)	GCAC	C	CGC-CT	CC	CGA	A--GCTG-C	C	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Manitoba (E128)	GCAC	C	CGC-CT	CT/C	CGA	A--GC-G-C	C	TCT	-A/T	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Minnesota (E134)	GCAC	C	CGC-CC/T	CC	CGA	A--GCTG-C	C/A	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
New Mexico (E97)	GCAC	C	CGC-CC/T	CC	CGA	A--GCTG-C	A	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Texas (E135)	GCAC	C	CGC-CC	CC	CGA	A--GCTG-C	C	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Utah (E136)	GCAC	C	CGC-CC	CC	CGA	T/A--GCTG-C	C	TCT	-T	CTCCG	CA---------AGGC	CAACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Québec (E137)	GCAC	C	CGC-CT	CC	CGA	A--GCTG-C	A/C	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Québec (E129)	GCAC	C	CGC-CT/C	CC	CGA	A--GCTG-C	A/C	TCT	-T	CTCCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
(clade D)																					
Alaska (E130)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Alaska (E139)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
California (E131)	TCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Idaho (E112)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	TTTCG	CA---------GGGC	CTACG	CCG	ATCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Illinois (E132)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
British Columbia (E93)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CTG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
British Columbia (E103)	GCAC	C	CGC-CC	CC	AGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	A/CTACG	CTG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Yakutia (E111)	GCAC	C	CGC-CC	CC	CGA	A--AC-G-C	C	TCT	-T	CTTCG	CA---------AGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Yakutia (E70)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Yakutia (E113)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	TTTCG	CA---------GGTC	CTACG	CCG	ATCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
Altai (E81)	GCAC	C	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC
W Siberia (E92)	GCAC	C/T	CGC-CC	CC	CGA	AGAGC-G-C	C	TCT	-T	CTTCG	CA---------GGGC	CTACG	CCG	TTCCTATCACTT	T	CCTATGAGGTG	A	GTGAGGCCT	CG	GC	CGC

(continued on next page)

Table 2 (*continued*)

(clade E)	
Altai (E57)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CT GA CGC
Altai (E58)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CT GA CGC
Altai (E85)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CT GA CGC
Altai (E95)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CT GA CGC
W Siberia (E59)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CT GA CGC
***Euconulus polygyratus* (clade F)**	
Iowa (E171)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Maine (E168)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Michigan (E170)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Minnesota (E169)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -A/TCTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
New York (E172)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Ohio (E167)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------ATGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Virginia (E153)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Ontario (E154)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT TT CTTCG CA---------AGGC CTACG CCG CTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
***Euconulus* aff. *alderi* (clade G)**	
Iowa (E117)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTAAG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT TG GC CGC
Maine (E118)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTAAG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT TG GC CGC
Michigan (E119)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTAAG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT TG GC CGC
Utah (E120)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTAAG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT TG GC CGC
British Columbia (E110)	GCAC C CGC-CC CC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTAAG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT TG GC CGC
***Euconulus* aff. *fulvus* (clade H)**	
Japan (E121)	GAAC A CGC-CC TC CGA A--GA-G-C C TCG/T-T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Japan (E123)	GAAC A CGC-CC TC CGA A--GA-G-C C TCT/G-T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Japan (E122)	GAAC A TGC-TC TC CGA A--AC-GGC C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Japan (E124)	GAAC A TGC-TC TC CGG/AA--AC-GGC C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Japan (E126)	GCAC C CGT-CC TC CGA A--GC-G-C C TCT -T CTTCG CA---------AGGC CTACG CCG TTCCTATCACTT T CCTATGAGGTG A GTGAGGCCT CG GC CGC
Outgroup taxa	
E. trochulus; Arkansas (E156)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CTTCG AGAATCGTCGGAGGC CTACG CCG TACCTATCACTT T CCT-TGCGGTG A GTGAGGA-T CG GC CGC
E. trochulus; N Carolina (E165)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CTTCG CAAATCGTCGGAGGC CTACG CCA/GTACCTATCACTT T CCT-CGCGGTG A GTGAGACCT CG GC AGC
E. trochulus; Oklahoma (E155)	GCGC C TGCACC CC CGA A--GA-A-C C TCT -T TTTCG CAAATCGTCGGAGGC CTACG CCG TACCTATCACTT T CCT-TGCGGTG A GTGAGACCT CG GC AGC
E. dentatus; Georgia (E157)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CTTCG CAAATCGTCGGAGGC CTACG CCG TACCTATCACTT T CCT-TGCGGTG A GTGAGGA-T CG GC CGC
E. dentatus; Mississippi (E158)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CTTCG CAAATCGTCGGAGGC CTACG CCG TACCTATCACTT T CCT-TGGGGTT/GT/AGTGAGGA-T CG GC CGC
E. dentatus; Virginia (E164)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CTTCG AAAATCGTCGGAGGC CTACG CCG TACCTATCACTT T CCT-TGGGGTT T GTGAGGA-T CG GC CGC
E. chersinus; Florida (E160)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CTACG CAAATCGTCGGAGGC CTGCG CCG TACCTATCACTT T CCT-CGCGGTG A GTGAGACCT CG GC AGC
E. chersinus; Florida (E166)	GCAC C CCCACC CC CGA A--GC-A-C C TCT -T CTACG CAAATCGTCGGAGGC CTGCG CCG TACCTATCACTT T CCT-CGCGGTG A GTGAGACCT CG GC AAC
E. chersinus; N Carolina (E159)	GCAC C CGCACC CC CGA A--GC-A-C C TCT -T CCTCG AAAATCGTCGGAGGA/CCTACG CCG TACCTATCACTT T CCT-TGGGGTT/GT/AGTGAGGA-T CG GC CGC

distributed (Fig. 3B), with the first ordination axis principally reflecting shell width and number of whorls ratio, and the second axis the shell height and shell width ratio (Table 3). In this ordination North American *E.* aff. *alderi* (clade G) was highly distinct from European/Beringian *E. alderi* (clade A) primarily due to a tighter coiling ratio. Additionally, European/Beringian *E. alderi* (clade A) differed from *E. f. fulvus* by its relatively more conical shells. Qualitative morphological features also separated these forms, with *E. alderi* having darker red-brown shells of shiny lustre, stronger bottom spiral grooves, darker body and possessing a uniform dark-grey mantle (Table 4). By comparison, North American *E.* aff. *alderi* (clade G) differed from *E. alderi* (clade A) in its mottled mantle and prominent keel (Table 4).

We were unable to observe any significant morphometric differentiation between *E. f. fulvus* and *E. f. egenus* clades B, C, D, E, and H. However, we did note some weak and oft-violated trends. For instance, whorl width expansion rate tended to be somewhat higher in clades C and D, although with a number of clade B and E individuals falling within this range. Additionally, protochonch/teleochonch

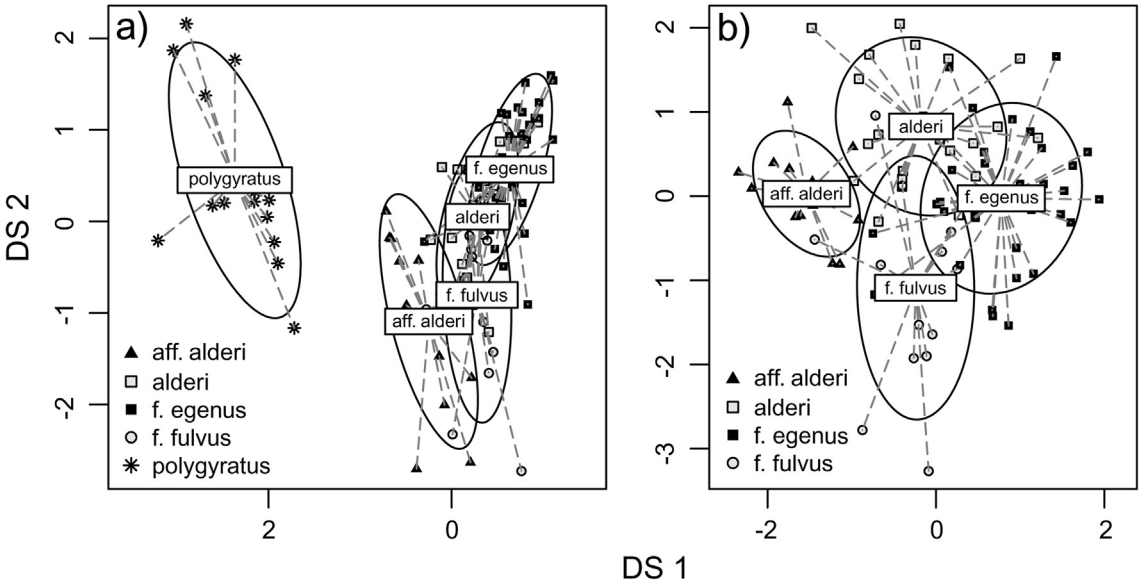

Fig. 3. Position of measured *Euconulus* shells along the first two axes of discriminant analysis based on seven shell characteristics (see Table 3). Ellipses show 0.95 confidential interval. The analysis was run based on all five taxa (A), and with *E. polygyratus* being excluded (B). Numbers of measured shells/populations: *E. alderi* (clade A) = 27/7, *E. fulvus fulvus* (clade B) = 16/6, *E. fulvus egenus* (clades C–E) = 35/12, *E. polygyratus* (clade F) = 14/4, *E.* aff. *alderi* (clade G) = 14/4.

Table 3
Multiple regressions of shell characteristics and specimen scores on the first two discriminant axes (DS 1 and DS 2). Regression coefficients; the fit of each shell characteristic into the ordination space, i.e. variation in the characteristic explained by specimen scores on the first two DS axes in multiple linear regression (r^2); and significance (p) of the result based on 4999 permutations are shown. The highest regression coefficients of significant variables ($p < 0.05$) are in bold. A, the analysis was run based on all five taxa; and B, with *E. polygyratus* being excluded. Not significant values are given in italics. *E.* aff. *fulvus* from Japan (clade H) was excluded from the analysis due to the lack of material of fully mature individuals.

	DS 1	DS 2	r^2 (%)	p
(A) All five taxa				
Shell width/no. of whorls	**−0.970**	0.244	99.0	< 0.001
Shell height/no. of whorls	−0.861	0.509	87.5	< 0.001
Shell height/shell width	0.798	0.602	55.1	< 0.001
Body whorl height/shell height	−0.856	−0.516	72.5	< 0.001
Aperture width/shell width	−0.939	0.343	40.5	< 0.001
Aperture width/shell height	−0.945	−0.328	63.8	< 0.001
Aperture height/body whorl height	−0.117	**0.993**	7.0	0.036
(B) Without E. polygyratus				
Shell width/no. of whorls	**0.995**	0.098	92.7	< 0.001
Shell height/no. of whorls	0.891	0.454	79.6	< 0.001
Shell height/shell width	0.073	**0.997**	35.3	< 0.001
Body whorl height/shell height	*−0.694*	*−0.720*	*2.7*	*0.323*
Aperture width/shell width	0.570	0.822	47.6	< 0.001
Aperture width/shell height	0.564	−0.826	12.3	0.004
Aperture height/body whorl height	0.890	−0.457	18.8	< 0.001

microsculpture tended to be stronger in clade D and weakest in clades B and E. Again, so much overlap was observed as to make this trait non-diagnostic. We also noted that *E. f. fulvus* tended to possess uniform pale mantle tissue while *E. f. egenus* was mottled. However, some high Alps populations of *E. f. fulvus* also exhibited mottled mantle coloration, while some *E. f. egenus* were observed to be uniform.

4. Discussion

4.1. Species concepts

Any consideration of species (and cryptic species) must explicitly consider which species concept is being applied (Denise et al., 2008). However, with over 20 different approaches having been elucidated (e.g. Mayden, 1997; De Queiroz, 2007; Zachos, 2016) this choice remains largely based on personal preference (e.g. Baker and Bradley, 2006; Cracraft, 1992; Mishler and Donoghue, 1982). Ours is to consider a species distinct once it has become a quasi-independent evolutionary unit. Although some suggest that any single line of evidence can form a basis of a species discovery (e.g. De Queiroz, 2007; Padial et al., 2010), we believe that this is not biologically reasonable and expect that almost all the taxa in question will be distinct across multiple data-streams. For terrestrial gastropods this includes some reasonable subset of mtDNA sequence, nDNA sequence, conchology, genital anatomy, ecological preference and/or biogeography. Only when a consensus for distinctness exists can we be soundly confident that a given entity has begun to take its own individual evolutionary path and represents a distinct species. Our approach is close to the 'evolutionary species concept' (Simpson, 1961) as well as to the 'biological species concept' (Mayr, 1942), however we note that ours is more statistically-focused with the simple appearance of fertile hybrids or genetic introgression to our mind not necessarily requiring the lumping of two entities, especially when such events are rare and consistent divergence is seen across a suite of other traits.

4.2. Species richness in Euconulus

There should be little debate over the species-level status of *E. alderi* (clade A), North American *E.* aff. *alderi* (clade G), and *E. polygyratus* (clade F). Each of these not only show similar distinct topological relationships between both mtDNA and nDNA sequence, but also demonstrate unique biogeography and ecological preferences in addition

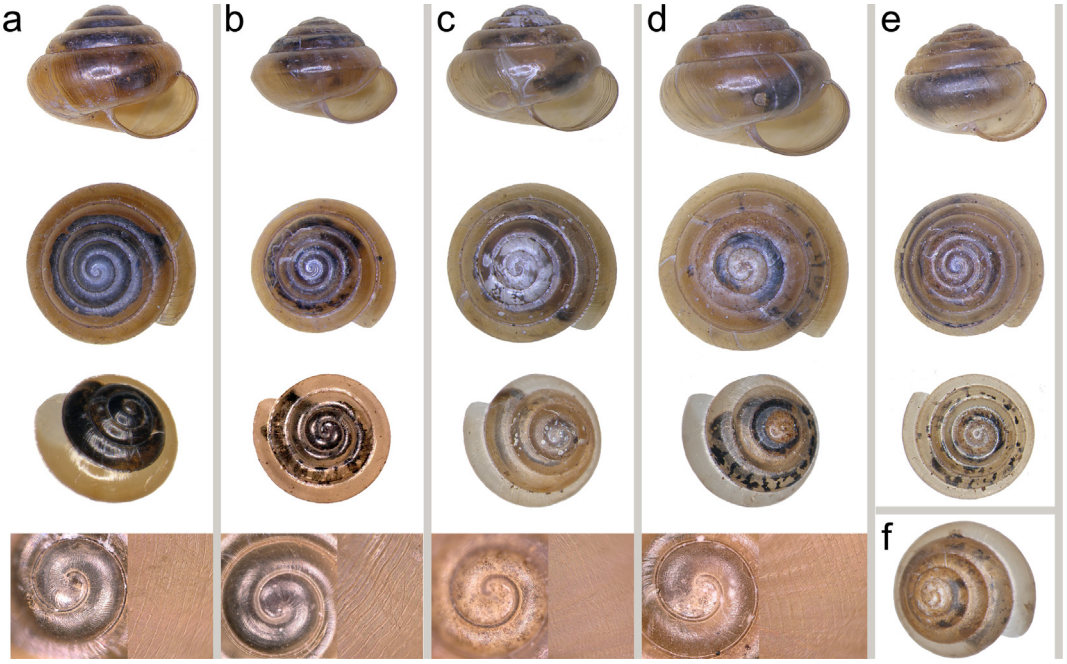

Fig. 4. Shell variation and main shell characters of studied *Euconulus* taxa: A, *E. alderi* (clade A), low marly meadow, Alaska, USA (E115), 3.08 × 2.42 mm; B, *E.* aff. *alderi* (clade G), grazed wet sedge mat, Iowa, USA (E117), 1.94 × 2.62 mm; C, *E. fulvus fulvus* (clade B), mountain Spruce forest, Moravia, Czech Republic (E83), 2.30 × 3.09 mm; D, *E. fulvus egenus* (clades C–E), *Salix* scrub, Western Siberia, Russia (E59), 2.72 × 3.44 mm; E, *E. polygyratus* (clade F), cool limestone bluff, Ohio, USA (E167), 2.75 × 2.18 mm; F, *E.* aff. *fulvus* (clade H), mesic grassland, Hokkaido, Japan (E124), 1.28 × 1.82 mm. Note that the measurements refer to the individuals shown in the first two rows and in (F) for *E.* aff. *fulvus* (clade H). Images shown in the third row show individuals which were used for the DNA extraction; other imaged individuals were selected from the same samples as those used for the DNA work.

Table 4
Variation in morphological characters of studied *Euconulus* taxa. *Euconulus* aff. *fulvus* from Japan (clade H) was excluded due to the lack of fully mature individuals.

Taxon	Max. no. of whorls	Shell width/No. of whorls (min–max)	Bottom spiral grooves	Body color	Shell color/lustre (from top)	Mantle	Keel on body whorl	Protoconch structure
E. alderi (clade A)	5.10	0.57–0.69	Strong sparse	Black	Brownish shell/glossy	Uniform dark-grey	Almost absent	Coarse growth lines
E.* aff. *alderi (clade G)	5.00	0.52–0.61	Strong sparse	Black	Brownish shell/glossy	Mottled	Prominent	Smooth to very weak
E. fulvus fulvus (clade B)	5.30	0.56–0.63	Weak dense (also moderate sparse*)	Pale	Yellowish/silky to dull (also glossy*)	Uniform pale (mottled*)	Almost absent	Strong to weak cross-like
E. fulvus egenus (clades C–E)	5.00	0.57–0.72	Weak dense to moderate sparse	Pale	Yellowish/silky to semi-glossy	Usually mottled	Absent to weak	Strong to weak cross-like
E. polygyratus (clade F)	6.60	0.41–0.46	Weak to absent	Pale	Yellowish/dull	Mottled	Weak	Smooth to very weak

* In high mountain wetland populations.

to a unique suite of shell traits/morphometrics: Only *E. alderi* has a dark animal with uniform dark-grey mantle possessing a dark brown shell with glassy lustre, a low coiling ratio, rounded whorl margin in adults, and reduced microsculpture on the protochonch/teleochonch. Only *E.* aff. *alderi* has a dark animal with mottled mantle possessing a dark brown shell with glassy lustre, a higher coiling ratio than *E. alderi*, keeled whorl margin in adults, and reduced microsculpture on the protochonch. Only *E. polygyratus* has a light animal with mottled mantle possessing a tan/yellow shell with a dull lustre, a large coiling ratio, weakly keeled whorl margin in adults, and reduced microsculpture on the protochonch/teleochonch. Neither mtDNA nor nDNA provide evidence of *E. alderi* being a sibling of *E.* aff. *alderi*. Thus even though they are morphologically (dark animals with dark brown, shiny shells and reduced protochonch/teleochonch sculpture) and ecologically (limited to wetlands) convergent, these two entities clearly represent separate species. Full species description (and name assignment) of *E.* aff. *alderi*, including a thorough taxonomic revision of the genus *Euconulus*, is currently being prepared (Horsáková et al. unpublished results).

The case for *E. fulvus* is not as clear. On one hand nDNA shows the existence of five separate clades, each with a rather circumscribed biogeographic range. It would thus seem reasonable to naively assume that *E. fulvus* represents a complex of five distinct allopatrically distributed cryptic species. We have carefully considered this approach, but decided to reject it for the following reasons: (1) Other than biogeography, we were unable to document any significant macroscopic trait differences between clades, with the total range of all observed features being seen within each clade. In and of itself this would not be enough to invalidate a cryptic species hypothesis given that by their very nature such species may not possess observable differences. However, we also note the following: (2) Considerable incongruence exists in placement of individuals between mtDNA and nDNA trees, especially among the *E. f. egenus* clades C, D and E. Clearly there has been – and likely continues to be – significant introgression and/or incomplete sorting between these entities. Moreover, node support for the three above clades was inconsistent, with neither of these clades reaching a threshold for a significant support in all the reconstruction methods. (3) We note the presence of frequent heterozygosity (i.e. occurrence of double peaks in the sequence chromatograms) within the nDNA sequence. This could potentially be attributed to several factors, e.g. a multi-copy character of genetic markers that are part of the ribosomal nDNA cluster (such as ITS), presence of pseudogenes, or simple sequencing errors based on unequal fluorophore signals. Nevertheless, the nature of the heterozygous positions in our data, being expressed as two virtually equal peaks (roughly a half of a proportion of other peaks) within a very clear chromatogram, strongly suggests that these are results of recent hybridization between two closely related individuals (e.g. Andersson, 2012; Huyse et al., 2009; Sonnenberg et al., 2007). Moreover, all the heterozygous positions combine only bases diagnosing various *Euconulus* clades, and not other random bases. The most striking example may be the co-dominance of nucleotides characterizing European (clade B) and central Asian (clade E) populations within some Scandinavian and central European individuals. This suggests hybridization between these two lineages within the relatively recent past – perhaps during the Last Glacial Maximum when a number of central Asian land snail species frequently occurred across the European steppe-tundra zone (Horsák et al., 2015). As a result of demonstrable evidence of frequent mtDNA/nDNA introgression, presumable hybridization, and lack of clear morphometric differences between groups we feel that empirical evidence is lacking to support evolutionary independence of the *E. fulvus* clades. We are thus not willing to consider them as representing distinct cryptic species at this time. If some of the genetic structure in *E. fulvus* is to be recognized, we suggest differentiating as subspecies the well-defined European *E. f. fulvus* from the less well sorted non-European *E. f. egenus*.

4.3. Macroevolutionary process in Euconulus

These data provide important macroevolutionary insights into diversification of *Euconulus*, suggesting both allopatric and sympatric processes being present. Allopatric processes are likely present in *E. fulvus* which is made up of five different relatively geographically distinct clades (Europe, Central Asia, East Asia, Beringia, and North America). This geography mirrors allopatric replacement patterns in a number of other Holarctic micro-snails, such as *Punctum*, *Pupilla* and *Vertigo* (Horsák and Meng, 2018; Nekola et al., 2015, 2018), suggesting a similar history of isolation and evolutionary diversification over the Pleistocene glacial/interglacial cycles. Even though we do not consider these *E. fulvus* genetic clades to represent distinct species, the divergent nature of their topology in nDNA suggests that they might be along the road to evolutionary independence. The current absence of effective dispersal barriers (such as continental ice sheets during the glacial periods), along with the ecological generalism of *E. fulvus*, is likely hindering allopatric speciation. The *E. f. fulvus/E. f. egenus* group thus appears to provide a unique window into macroevolutionary allopatric process.

Sympatric processes among species originating from different common ancestors on different continents yet driven by similar selective pressures (e.g. Trontelj and Fišer, 2009) appear likely for *E. alderi* and *E.* aff. *alderi*. Although the ancestor-descendant relationships among the studied *Euconulus* species could not be resolved based on our data, the above hypothesis can be corroborated by the shell features of some *E. f. fulvus* individuals. Several high-mountain European populations of this species also possess shinier shells and darker (i.e. mottled) mantles compared to lowland (although genetically identical) *E. f. fulvus* populations. Additionally we noted some *E. f. egenus* populations from cool, humid algific talus slope sites in the Upper Mississippi Valley to show some convergence with *E.* aff. *alderi* in terms of reduced microsculpture and lower rates of whorl width expansion as compared to typical upland populations. We have noted similar features in wetland-restricted species of other micro-snail genera (e.g. *Pupilla*, *Strobilops*, *Vertigo*, and *Zonitoides*), but the root cause of this pattern remains unresolved. The environment-dependent morphology in European *E. f. fulvus* populations highlights a high potential for phenotypic plasticity of traits that were traditionally used to distinguish *Euconulus* species. A reliance on plastic shell features likely led to a taxonomic confusion also in North American *E. chersinus*, *E. dentatus* and *E. trochulus*, which were used as outgroup taxa in our study and which genetically deviated from their morphology-based *a priori* assignments.

4.4. When a lineage should be considered a cryptic species

For the cryptic species that we introduce here, i.e. North American *E.* aff. *alderi* (clade G), a firm corroboration was achieved from nDNA, mtDNA and multiple quantitative and qualitative shell characteristics including differential coiling ratios, prominence of a keel, and presence/absence of a mottled mantle. As such, *E.* aff. *alderi* meets the definition of a "pseudo-cryptic" species (Sáez and Lozano, 2005). However, we prefer avoiding this term, as it may potentially introduce confusion regarding how pronounced and consistent the morphological differences must be to elevate the species from a cryptic to a "pseudo-cryptic" status, or whether these differences are so pronounced to negate the "crypticity" at all. After all, all cryptic species, once introduced, have a high potential for a later discovery of characteristic traits (Korshunova et al., 2017).

Our second example of *E. fulvus* clades B–H might be suggestive of a complex of "fully cryptic" species. Proponents of integrative taxonomy emphasize a multi-source approach of complementary evidence from various disciplines to achieve rigorous species delimitations (e.g. Dayrat, 2005; Will et al., 2005; Schlick-Steiner et al., 2010), such as accomplished for *E.* aff. *alderi* in our study. For *E. f. fulvus* and *E. f. egenus*, no observable diagnostic differences could be found in any quantitative or qualitative trait between any of the five clades, and with the full range of values being present within each clade for every investigated trait. Of course, we can only speak about the factors that we observed, and it is possible that some unobserved trait could provide such distinction (Sáez and Lozano, 2005). But importantly we could not find these, even though we investigated most of the possible different macro-scale features. The preponderance of data thus seems to suggest that for all intents the shells of these five clades are indistinguishable. On the other hand, some authors suggest that any single line of evidence (e.g. DNA data) can document a species existence if the support for lineage divergence is robust enough (e.g. Valdecasas et al., 2007; Padial and De la Riva, 2010; Jörger and Schrödl, 2013; Fišer et al., 2018). With this respect, we suggest two criteria that need to be met to propound a hypothesis of molecular-based "fully cryptic" species. First, cryptic entities should be separated from described forms by highly supported reciprocally monophyletic clades in multiple independent genetic markers. Ideally, both nDNA and mtDNA should be consulted (Rubinoff and Holland, 2005). This condition also means that little incongruence exists between placement of individuals between well-supported clades. If clades have become independent evolutionary units, then topological assignments should be relatively consistent between various datastreams. We are aware that the evolutionary processes are not straightforward and that discordance between multiple genetic loci is a widespread phenomenon (Degnan and Rosenberg, 2009). However, in cases when other evidence is lacking, entering into a cryptic species hypothesis would rarely be justifiable if molecular markers fail to be congruent. In the *Euconulus* example above, *E. alderi* vs. *E.* aff. *alderi* demonstrated no instances of topological incongruence in either dataset while 25% of *E. f. egenus* individuals demonstrated incongruence in their assignment between nDNA and mtDNA. Second, we suggest that little evidence exists for hybridization between cryptic clades. The presence of hybrid individuals (suggested by mixed bases characteristic of two different cryptic clades in the nDNA chromatograms) indicates that genetic interchange is still ongoing. Although interbreeding and gene flow do not necessarily preclude speciation (Hausdorf, 2011), we argue that they should be most critically considered when other than molecular evidence for lineage divergence is lacking. In accordance with this, there was no indication of hybridization between *E. alderi* and *E.* aff. *alderi*, while in *E. fulvus*, two widely spaced individuals (one from Scandinavia, the other from Central Europe) both demonstrated seven different positions which harbored nucleotides characteristic of both clades B and E.

However, we cannot stress enough that even when the above criteria are met, it only implies that the cryptic species hypothesis is possible, but should optimally be subjected to further examination as the observed genetic differences may only reflect a population structure of a single species (e.g. Bickford et al., 2007; Pinceel et al., 2004; Tan et al., 2010). The essential requirement should be to compare the extent of genetic differentiation among the presumably cryptic species with their closely related non-cryptic relatives (Struck et al., 2018), but the integration and cross-validation of multiple data streams still represents the most reliable taxonomic praxis. Species separation requires gene flow interruption, and therefore must be grounded in macro-scale biological differences/processes (e.g. morphology, anatomy, ecology, behavior). If species are indeed taking their own independent evolutionary walks, then truly "cryptic" species should be exceedingly rare. Rather what "cryptic" usually means is that we as humans have simply missed the features that actually exist, and that these entities are "cryptic" only from our naive frame of reference. Thus, "cryptic" says more about human perception than it does about biology.

We conclude that a cryptic species may represent a valid and useful taxonomic construct, but argue that entering into a cryptic species hypothesis is a responsible task that should not be taken lightly in modern phylogenetic studies. Considering the magnitude of ongoing habitat loss and anthropogenic interference with the environment, accurate estimations of Earth's species diversity are essential, yet heavily

reliant on justifiable recognitions and descriptions of cryptic species. Only if we are able to correctly detect such species will we succeed to draw firm conclusions in all fields of biology, including future predictions of ecosystem changes and biodiversity conservation.

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Appendix A. Taxon name, habitat information, sample code and GenBank accession number for each of the analyzed *Euconulus* specimens

Taxon/Country	State/Province/ Region	Habitat	Latitude °N	Longitude °E	Sample code	GenBank accession number			
						16S	COII	ITS1	ITS2
Euconulus alderi (clade A)									
Czech Republic	Moravia	Wet meadow	48.8068	16.8382	E109	MK266536	MK299609	MK299792	MK299699
Czech Republic	Moravia	*Sphagno-Tomentypnion* fen	49.7191	16.1251	E71	MK266512	MK299585	MK299768	MK299675
Czech Republic	Moravia	Wet alder forest on a spring	49.1198	17.0412	E91	MK266526	MK299599	MK299782	MK299689
Czech Republic	Český les	Willow shrubs on a fen margin	49.6984	12.4728	E45	MK266498	MK299571	MK299754	MK299661
Slovakia	Levoča Mts.	Brown-moss rich fen	49.2052	20.7865	E41	MK266495	MK299568	MK299751	MK299658
Slovakia	Danube plateau	Reed-sedge wetland	47.8740	17.6709	E76	MK266515	MK299588	MK299771	MK299678
Austria	Niederösterreich	Wet brown-moss rich fen	47.8238	15.4713	E40	MK266494	MK299567	MK299750	MK299657
Poland	Orava	Brown-moss rich fen	49.4684	19.8196	E68	MK266510	MK299583	MK299766	MK299673
Poland	Lubelskie	Wet brown-moss rich fen	51.3452	23.3371	E86	MK266521	MK299594	MK299777	MK299684
Poland	Podlaskie	Wet brown-moss rich fen	53.9041	22.9534	E94	MK266529	MK299602	MK299785	MK299692
Sweden	Jämtland	Wet calcareous fen	63.4154	14.5544	E75	MK266514	MK299587	MK299770	MK299677
Sweden	Jämtland	Wet calcareous fen	63.5802	15.2311	E61	MK266504	MK299577	MK299760	MK299667
Switzerland	Fribourg	Rich fen on a lake margin	46.8379	6.8126	E2	MK266493	MK299566	MK299749	MK299656
Switzerland	Graubünden	Brown-moss rich fen	46.7772	10.2821	E72	MK266513	MK299586	MK299769	MK299676
Romania	Harghita	Calcareous fen	47.0784	25.4777	E88	MK266523	MK299596	MK299779	MK299686
Bulgaria	Bulgaria	Brown-moss rich fen	42.7064	24.1117	E89	MK266524	MK299597	MK299780	MK299687
USA	Alaska	Low, marly meadow	67.4775	−149.917	E115	MK266541	MK299614	MK299798	MK299705
USA	California	*Juncus-Carex-Geum* wet meadow	38.2253	−119.250	E116	MK266542	MK299615	MK299799	MK299706
Russia	Western Siberia	*Salix lapponum-S. phylicifolia* scrub	66.4442	79.3228	E56	MK266500	MK299573	MK299756	MK299663
Euconulus* aff. *alderi (clade G)									
Canada	British Columbia	Extremely rich fen in river alluvium	50.9213	−117.577	E110	MK266537	MK299610	MK299793	MK299700
USA	Iowa	Heavily grazed wet sedge mat	42.0406	−91.3264	E117	MK266543	MK299616	MK299800	MK299707
USA	Maine	Rich cedar-ash-maple swamp	44.9272	−67.6589	E118	MK266544	MK299617	MK299801	MK299708
USA	Michigan	Rich marly sedge mat	42.4306	−83.9792	E119	MK266545	MK299618	MK299802	MK299709
USA	Utah	Calcareous seep	37.3742	−112.594	E120	MK266546	MK299619	MK299803	MK299710
Euconulus fulvus fulvus (clade B)									
Czech Republic	Krušné hory Mts.	Seepage in Ash forest	50.6673	13.6708	E80	MK266516	MK299589	MK299772	MK299679
Czech Republic	Moravia	Mountain deciduous forest	48.8550	17.6690	E82	MK266518	MK299591	MK299774	MK299681
Czech Republic	Moravia	Mountain spruce forest	49.5047	18.3761	E83	MK266519	MK299592	MK299775	MK299682
Slovakia	Kysuce NR	Rich fen with willows	49.4256	18.5255	E63	MK266506	MK299579	MK299762	MK299669
Poland	Orava	Fen meadow on a slope	49.3369	19.9055	E87	MK266522	MK299595	MK299778	MK299685
Austria	Niederösterreich	Wet brown-moss rich sloping fen	47.8514	15.3895	E42	MK266496	MK299569	MK299752	MK299659
Switzerland	Valais	Brown-moss rich fen on a brook margin	45.9990	7.7551	E1	MK266492	MK299565	MK299748	MK299655
Switzerland	Valais	Rich fen on a spring	46.0018	7.3407	E62	MK266505	MK299578	MK299761	MK299668
Switzerland	Valais	Sloping calcareous fen	46.0072	7.7934	E50	MK266499	MK299572	MK299755	MK299662
Switzerland	Bern	Sloping calcareous fen with *Schoenus*	46.5606	7.0769	E64	MK266507	MK299580	MK299763	MK299670
Switzerland	Graubünden	Brown-moss rich fen	46.6741	10.3523	E67	MK266509	MK299582	MK299765	MK299672
Sweden	Jämtland	*Sphagno-Tomentypnion* fen	63.5802	15.2311	E101	MK266534	MK299607	MK299790	MK299697
Sweden	Jämtland	Sloping brown-moss rich fen	63.5684	12.2458	E66	MK266508	MK299581	MK299764	MK299671
Austria	Salzburg	Limestone slope	47.2334	13.5067	E98	MK266532	MK299605	MK299788	MK299695
Norway	Dovrefjell NP	Willow shrubs on a brook margin	62.3548	9.6702	E90	MK266525	MK299598	MK299781	MK299688
Romania	Harghita	Wet brown-moss rich fen	46.3176	25.5999	E44	MK266497	MK299570	MK299753	MK299660
Iceland	Mývatn	Willow tundra	65.6289	−16.9928	E161	MK266571	MK299644	MK299829	MK299736
Iceland	Skaftafell NP	Willow tundra	64.0264	−16.9779	E162	MK266572	MK299645	MK299830	MK299737
Iceland	Skaftafell NP	Willow tundra	64.0264	−16.9779	E163	MK266573	MK299646	MK299831	MK299738
Russia	Western Siberia	Mesic birch forest	56.5067	68.4156	E99	MK266533	MK299606	MK299789	MK299696
Euconulus fulvus egenus (clades C,D,E)									
USA	Idaho	Douglas fir forest at base of open talus	47.6477	−115.972	E112	MK266539	MK299612	MK299795	MK299702
USA	California	Damp creekside with fern	34.1792	−116.906	E131	MK266555	MK299628	MK299812	MK299719

USA	Illinois	East-facing limestone cliff	42.2806	−89.3686	E132			MK299813	MK299720
USA	Maine	Rich thuja-ash-red maple swamp	44.9272	−67.6589	E133	MK266556	MK299629	MK299814	MK299721
USA	Minnesota	Aspen-ash-balsam-spruce forest	47.6211	−95.3056	E134	MK266557	MK299630	MK299815	MK299722
USA	Texas	Dry rocky oak-juniper forest	29.2442	−103.297	E135	MK266558	MK299631	MK299816	MK299723
USA	Utah	Aspen fringe on S-facing talus slope	38.4169	−112.313	E136	MK266559	MK299632	MK299817	MK299724
USA	New Mexico	Pine-fir forest	35.7494	−105.659	E97	MK266531	MK299604	MK299787	MK299694
USA	Alaska	Wet mesic alder-aspen-birch forest	64.6064	−149.090	E138	MK266561	MK299634	MK299819	MK299726
USA	Alaska	Rich upland tundra	69.3353	−148.730	E139	MK266562	MK299635	MK299820	MK299727
USA	Alaska	Spruce-alder streamside	60.9750	−149.121	E130	MK266554	MK299627	MK299811	MK299718
Canada	Québec	Willow scrub on tundra	55.0644	−67.2347	E137	MK266560	MK299633	MK299818	MK299725
Canada	Québec	Rich aspen-maple-birch forest	49.3256	−67.3700	E129	MK266553	MK299626	MK299810	MK299717
Canada	British Columbia	Extremely rich fen in river alluvium	50.9213	−117.577	E103	MK266535	MK299608	MK299791	MK299698
Canada	British Columbia	Willow shrubs with shist scree	50.6368	−117.192	E93	MK266528	MK299601	MK299784	MK299691
Canada	Labrador	Rich *Carex-Calamagrostis* turf	54.6725	−66.6075	E114	MK266540	MK299613	MK299797	MK299704
Canada	Labrador	Rich mature spruce-fir forest	54.6725	−66.6075	E127	MK266552	MK299625		
Canada	Manitoba	Brushy willow-spruce tundra	58.7514	−93.9139	E128			MK299809	MK299716
Russia	Altai	*Betula* scrub on boulder accumulation	49.6288	87.6569	E57	MK266501	MK299574	MK299757	MK299664
Russia	Altai	Fen shrubland in a floodplain	51.1146	85.5966	E58	MK266502	MK299575	MK299758	MK299665
Russia	Altai	Lake margin	50.47417	87.63444	E81	MK266517	MK299590	MK299773	MK299680
Russia	Altai	Hemiboreal forest	51.7339	85.7233	E85	MK266520	MK299593	MK299776	MK299683
Russia	Altai	Small woodlot	49.6426	87.8404	E95	MK266530	MK299603	MK299786	MK299693
Russia	Western Siberia	Salix scrub	67.1814	78.8589	E59	MK266503	MK299576	MK299759	MK299666
Russia	Western Siberia	Mesic pine-birch forest	63.4425	74.6075	E92	MK266527	MK299600	MK299783	MK299690
Russia	Yakutia	Pine taiga forest	62.5671	130.5288	E70	MK266511	MK299584	MK299767	MK299674
Russia	Yakutia	Birch-larch taiga	62.2821	129.7564	E111	MK266538	MK299611	MK299794	MK299701
Russia	Yakutia	Pine-spruce taiga	61.6531	129.2608	E113			MK299796	MK299703
Euconulus* aff. *fulvus (clade H)									
Japan	Hokkaido	Wet reed-sedge cover under ash	43.92	144.1586	E121	MK266547	MK299620	MK299804	MK299711
Japan	Hokkaido	Old field with lupine and willow	43.9335	144.4439	E122	MK266548	MK299621	MK299805	MK299712
Japan	Hokkaido	Sweetgum-magnolia-birch-fir forest	42.9517	144.7370	E123	MK266549	MK299622	MK299806	MK299713
Japan	Hokkaido	Mesic grassland on dune shore	42.5877	143.5358	E124	MK266550	MK299623	MK299807	MK299714
Japan	Tokyo	*Phragmites-Typha* seep	35.6325	139.4677	E126	MK266551	MK299624	MK299808	MK299715
Euconulus polygyratus (clade F)									
USA	Virginia	Mesic maple-elm forest	38.0631	−79.8883	E153	MK266563	MK299636	MK299821	MK299728
USA	Ohio	Cool limestone bluff with white cedar	39.7953	−83.8364	E167	MK266576	MK299649	MK299835	MK299742
USA	Maine	Upland maple-birch-beech forest	46.0444	−68.1722	E168	MK266577	MK299650	MK299836	MK299743
USA	Minnesota	Mesic rocky forest	43.7221	−91.6420	E169	MK266578	MK299651	MK299837	MK299744
USA	Michigan	Rich rocky N-facing wooded slope	45.9077	−84.7470	E170	MK266579	MK299652	MK299838	MK299745
USA	Iowa	N-facing algific slope and cliff	43.1373	−91.4782	E171	MK266580	MK299653	MK299839	MK299746
USA	New York	Forest on limestone pavement	43.0074	−76.1105	E172	MK266581	MK299654	MK299840	MK299747
Canada	Ontario	Maple forest on limestone outcrop	44.9264	−75.7594	E154	MK266564	MK299637	MK299822	MK299729
Euconulus trochulus (outgroup)									
USA	Oklahoma	Mesic wooded sandstone outcrops	36.0131	−96.9972	E155	MK266565	MK299638	MK299823	MK299730
USA	Arkansas	N-facing wooded limestone bluff	35.9581	−92.1778	E156	MK266566	MK299639	MK299824	MK299731
USA	North Carolina	Medium pocosin wetland	34.9353	−77.0706	E165	MK266575	MK299648	MK299833	MK299740
Euconulus dentatus (outgroup)									
USA	Georgia	Forest margin	33.5433	−82.2542	E157	MK266567	MK299640	MK299825	MK299732
USA	Mississippi	Oak-bay litter on streamside	31.5039	−88.9253	E158	MK266568	MK299641	MK299826	MK299733
USA	Virginia	Dry W-facing limestone cliff	37.8222	−79.4325	E164	MK266574	MK299647	MK299832	MK299739
Euconulus chersinus (outgroup)									
USA	North Carolina	Oak logs in upland forest	35.2992	−81.1200	E159	MK266569	MK299642	MK299827	MK299734
USA	Florida	Virgin oak-hickory hammock	27.4692	−81.5489	E160	MK266570	MK299643	MK299828	MK299735
USA	Florida	Hardwood swamp	30.5353	−82.5600	E166			MK299834	MK299741

References

Agapow, P.M., Bininda-Emonds, O.R., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C., Purvis, A., 2004. The impact of species concept on biodiversity studies. Quart. Rev. Biol. 79, 161–179.

Andersson, J.O., 2012. Double peaks reveal rare diplomonad sex. Trends Parasitol. 28, 46–52.

Armbruster, G.F., Bernhard, D., 2000. Taxonomic significance of ribosomal ITS-1 sequence markers in self-fertilizing land snails of *Cochlicopa* (Stylommatophora, Cochlicopidae). Zoosyst. Evol. 76, 11–18.

Baker, R.J., Bradley, R.D., 2006. Speciation in mammals and the genetic species concept. J. Mammal. 87, 643–662.

Beheregaray, L.B., Caccone, A., 2007. Cryptic biodiversity in a changing world. J. Biol. 6, 9.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K., Meier, R., Winker, K., et al., 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22, 148–155.

Cracraft, J., 1992. The species of the birds-of-paradise (Paradisaeidae): applying the phylogenetic species concept to a complex pattern of diversification. Cladistics 8, 1–43.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Dayrat, B., 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 85, 407–415.

Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24, 332–340.
Denise, T.S.H., Ali, F., Kutty, S.N., Meier, R., 2008. The need for specifying species concepts: how many species of silvered langurs *(Trachypithecus cristatus* group) should be recognized? Mol. Phylogenet. Evol. 49, 688–689.
Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists. J. Stat. Softw. 22, 1–20.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Emberton, K.C., 1995. Cryptic, genetically extremely divergent, polytypic, convergent, and polymorphic taxa in Madagascan *Tropidophora* (Gastropoda: Pomatiasidae). Biol. J. Linn. Soc. 55, 183–208.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Fišer, C., Robinson, C.T., Malard, F., 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27, 613–635.
Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. Cladistics 24, 774–786.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52, 696–704.
Haase, M., Misof, B., 2009. Dynamic gastropods: stable shell polymorphism despite gene flow in the land snail *Arianta arbustorum*. J. Zool. Syst. Evol. Res. 47, 105–114.
Hausdorf, B., 2011. Progress toward a general species concept. Evolution 65, 923–931.
Heath, T.A., Hedtke, S.M., Hillis, D.M., 2008. Taxon sampling and the accuracy of phylogenetic analyses. J. Syst. Evol. 46, 239–257.
Heethoff, M., 2018. Cryptic species–conceptual or terminological chaos? A response to Struck et al. Trends Ecol. Evol. 33, 310.
Hillis, D.M., 1987. Molecular versus morphological approaches to systematics. Annu. Rev. Ecol. Syst. 18, 23–42.
Horsák, M., Juřičková, L., Picka, J., 2013. Molluscs of the Czech and Slovak Republics. Kabourek, Zlín.
Horsák, M., Chytrý, M., Hájková, P., Hájek, M., Danihelka, J., Horsáková, V., et al., 2015. European glacial relict snails and plants: environmental context of their modern refugial occurrence in southern Siberia. Boreas 44, 638–657.
Horsák, M., Meng, S., 2018. *Punctum lozeki* n. sp. – a new minute land-snail species (Gastropoda: Punctidae) from Siberia and Alaska. Malacologia 62, 11–20.
Horvath, C.D., 1997. Discussion: phylogenetic species concept: pluralism, monism, and history. Biol. Philos. 12, 225–232.
Hubricht, L., 1985. The distributions of the native land mollusks of the eastern United States. Fieldiana 24, 1–191.
Hugall, A., Moritz, C., Moussalli, A., Stanisic, J., 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc. Natl. Acad. Sci. 99, 6112–6117.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Huyse, T., Webster, B.L., Geldof, S., Stothard, J.R., Diaw, O.T., Polman, K., Rollinson, D., 2009. Bidirectional introgressive hybridization between a cattle and human schistosome species. PLoS Pathog. 5, e1000571.
Jörger, K.M., Schrödl, M., 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. Front. Zool. 10, 59.
Kerney, M.P., Cameron, R.A.D., 1979. Field Guide to the Land Snails of Britain and North-west Europe. Collins, UK.
Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420, 73–90.
Köhler, F., Johnson, M.S., 2012. Species limits in molecular phylogenies: a cautionary tale from Australian land snails (Camaenidae: *Amplirhagada* Iredale, 1933). Zool. J. Linn. Soc. 165, 337–362.
Köhler, F., Burghardt, I., 2016. Cryptic diversity in a widespread land snail: revision of the genus *Xanthomelon* Martens, 1860 from the Australian Monsoon Tropics (Pulmonata, Camaenidae). Zoolog. Scr. 45, 127–144.
Korshunova, T., Martynov, A., Bakken, T., Picton, B., 2017. External diversity is restrained by internal conservatism: new nudibranch mollusc contributes to the cryptic species problem. Zoolog. Scr. 46, 683–692.
de León, G.P.P., Nadler, S.A., 2010. What we don't recognize can hurt us: a plea for awareness about cryptic species. J. Parasitol. 96, 453–464.
Mayden, R.L., 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge, M.F., Dawah, H.A., Wilson, M.R. (Eds.), Species: The Units of Diversity. Chapman & Hall, pp. 381–423.
Mayr, E., 1942. Systematics and the Origin of Species from the Viewpoint of a Zoologist. Columbia University Press, New York.
Mishler, B.D., Donoghue, M.J., 1982. Species concepts: a case for pluralism. Syst. Zool. 31, 491–503.
Myšák, J., Horsák, M., Svobodová, E., Cernohorsky, N., 2013. Small-scale distribution of terrestrial snails: patterns of species richness and abundance related to area. J. Molluscan Stud. 79, 118–127.
Nekola, J.C., 2003. Terrestrial gastropod fauna of northeastern Wisconsin and the southern Upper Peninsula of Michigan. Am. Malacol. Bull. 18, 21–44.
Nekola, J.C., 2014. North American terrestrial gastropods through each end of a spyglass. J. Molluscan Stud. 80, 238–248.
Nekola, J.C., Coles, B.F., Bergthorsson, U., 2009. Evolutionary pattern and process within the *Vertigo gouldii* (Mollusca: Pulmonata, Pupillidae) group of minute North American land snails. Mol. Phylogenet. Evol. 53, 1010–1024.
Nekola, J.C., Coles, B.F., Horsák, M., 2015. Species assignment in *Pupilla* (Gastropoda: Pulmonata: Pupillidae): integration of DNA-sequence data and conchology. J. Molluscan Stud. 81, 196–216.
Nekola, J.C., Chiba, S., Coles, B.F., Drost, C.A., von Proschwitz, T., Horsák, M., 2018. A phylogenetic overview of the genus *Vertigo* O. F. Müller, 1773 (Gastropoda: Pulmonata: Pupillidae: Vertigininae). Malacologia 62, 21–161.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al., 2017. vegan: Community Ecology Package. R package version 2.4-3. <https://CRAN.R-project.org/package=vegan>.
Padial, J.M., De la Riva, I., 2010. A response to recent proposals for integrative taxonomy. Biol. J. Linn. Soc. 101, 747–756.
Padial, J.M., Miralles, A., De la Riva, I., Vences, M., 2010. The integrative future of taxonomy. Front. Zool. 7, 16.
Palumbi, S.R., Martin, A.P., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 2002. The Simple Fool's Guide to PCR. Department of Zoology Special Publication, University of Hawaii, Honolulu.
Pilsbry, H.A., 1946. Land Mollusca of North America (North of Mexico). Vol. 2, Part 1. The Academy of Natural Sciences of Philadelphia, Philadelphia.
Pinceel, J., Jordaens, K., Van Houtte, N., De Winter, A.J., Backeljau, T., 2004. Molecular and morphological data reveal cryptic taxonomic diversity in the terrestrial slug complex Arion subfuscus/fuscus (Mollusca, Pulmonata, Arionidae) in continental north-west Europe. Biol. J. Linn. Soc. 83, 23–38.
Pokryszko, B.M., Cameron, R.A.D., 2005. Geographical variation in the composition and richness of forest snail faunas in northern Europe. Rec. West. Aust. Mus., Suppl. 68, 115–132.
Pokryszko, B.M., Auffenberg, K., Hlaváč, J.Č., Naggs, F., 2009. Pupilloidea of Pakistan (Gastropoda: pulmonata): Truncatellininae, Vertigininae, Gastrocoptinae, Pupillinae (In Part). Annales Zoologici 59, 423–458.
De Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879–886.
R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, AT.
Rach, J., DeSalle, R., Sarkar, I.N., Schierwater, B., Hadrys, H., 2008. Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. Proc. R. Soc. Lond. B: Biol. Sci. 275, 237–247.
Raith, M., Zacherl, D.C., Pilgrim, E.M., Eernisse, D.J., 2016. Phylogeny and species diversity of Gulf of California oysters (Ostreidae) inferred from mitochondrial DNA. Am. Malacol. Bull. 33, 263–283.
Rees, W.J., 1965. The aerial dispersal of Mollusca. J. Molluscan Stud. 36, 269–282.
Rubinoff, D., Holland, B.S., 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Syst. Biol. 54, 952–961.
Sáez, A.G., Lozano, E., 2005. Body doubles. Nature 433, 111.
Schilthuizen, M., Cabanban, A.S., Haase, M., 2005. Possible speciation with gene flow in tropical cave snails. J. Zool. Syst. Evol. Res. 43, 133–138.
Schlick-Steiner, B.C., Seifert, B., Stauffer, C., Christian, E., Crozier, R.H., Steiner, F.M., 2007. Without morphology, cryptic species stay in taxonomic crypsis following discovery. Trends Ecol. Evol. 22, 391–392.
Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E., Crozier, R.H., 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu. Rev. Entomol. 55, 421–438.
Simison, W.B., Lindberg, D.R., 1999. Morphological and molecular resolution of a putative cryptic species complex: a case study of *Notoacmea fascicularis* (Menke, 1851) (Gastropoda: Patellogastropoda). J. Molluscan Stud. 65, 99–109.
Simpson, G.G., 1961. Principles of Animal Taxonomy. Columbia University Press, New York.
Soltis, P.S., Gitzendanner, M.A., 1999. Molecular systematics and the conservation of rare species. Conserv. Biol. 13, 471–483.
Sonnenberg, R., Nolte, A.W., Tautz, D., 2007. An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. Front. Zool. 4, 6.
Stamatakis, A., 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, open access: <http://bioinformatics.oxfordjournals.org/content/early/2014/01/21/bioinformatics.btu033.abstract?keytype=ref&ijkey=VTEqgUJYCDcf0kP>.
Struck, T.H., Feder, J.L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V.I., et al., 2018. Finding evolutionary processes hidden in cryptic species. Trends Ecol. Evol. 33, 153–163.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Tan, D.S., Ang, Y., Lim, G.S., Ismail, M.R.B., Meier, R., 2010. From 'cryptic species' to integrative taxonomy: an iterative process involving DNA sequences, morphology, and behaviour leads to the resurrection of *Sepsis pyrrhosoma* (Sepsidae: Diptera). Zoolog. Scr. 39, 51–61.
Teshima, H., Davison, A., Kuwahara, Y., Yokoyama, J., Chiba, S., Fukuda, T., et al., 2003. The evolution of extreme shell shape variation in the land snail *Ainohelix editha*: a phylogeny and hybrid zone analysis. Mol. Ecol. 12, 1869–1878.
Tongkerd, P., Lee, T., Panha, S., Burch, J.B., Foighil, D.Ó., 2004. Molecular phylogeny of certain Thai gastrocoptine micro land snails (Stylommatophora: Pupillidae) inferred from mitochondrial and nuclear ribosomal DNA sequences. J. Molluscan Stud. 70, 139–147.
Trontelj, P., Fišer, C., 2009. Perspectives: cryptic species diversity should not be trivialised. Syst. Biodivers. 7, 1–3.
Trontelj, P., Douady, C.J., Fišer, C., Gibert, J., Gorički, Š., Lefébure, T., et al., 2009. A molecular test for cryptic diversity in ground water: how large are the ranges of macro-stygobionts? Freshw. Biol. 54, 727–744.
Valdecasas, A.G., Williams, D., Wheeler, Q.D., 2007. 'Integrative taxonomy' then and now: a response to Dayrat (2005). Biol. J. Linn. Soc. 93, 211–216.
Wada, S., Kawakami, K., Chiba, S., 2012. Snails can survive passage through a bird's digestive system. J. Biogeogr. 39, 69–73.
Wada, S., Kameda, Y., Chiba, S., 2013. Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. Mol. Ecol. 22, 4801–4810.

Wade, C.M., Mordan, P.B., 2000. Evolution within the gastropod molluscs; using the ribosomal RNA gene-cluster as an indicator of phyolgenetic relationships. J. Molluscan Stud. 66, 565–569.
Weigand, A.M., Jochum, A., Slapnik, R., Schnitzler, J., Zarza, E., Klussmann-Kolb, A., 2013. Evolution of microgastropods (Ellobioidea, Carychiidae): integrating taxonomic, phylogenetic and evolutionary hypotheses. BMC Evol. Biol. 13, 18.
Welter-Schultes, F.W., 2012. European Non-Marine Molluscs, a Guide for Species Identification: Bestimmungsbuch für europäische Land-und Süsswassermollusken. Planet Poster Editions.
Will, K.W., Mishler, B.D., Wheeler, Q.D., 2005. The perils of DNA barcoding and the need for integrative taxonomy. Syst. Biol. 54, 844–851.
Wray, C.G., Landman, N.H., Saunders, W.B., Bonacum, J., 1995. Genetic divergence and geographic diversification in Nautilus. Paleobiology 21, 220–228.
Zachos, F.E., 2016. Tree thinking and species delimitation: guidelines for taxonomy and phylogenetic terminology. Mammalian Biol. – Zeitschrift für Säugetierkunde 81, 185–188.
Zielske, S., Haase, M., 2015. Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae). Mol. Phylogenet. Evol. 89, 171–181.