Integrative taxonomic consideration of the Holarctic *Euconulus fulvus* group of land snails (Gastropoda, Stylommatophora)

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While among the most common Holarctic land snails, species of the *Euconulus fulvus* group have been subject to considerable recent taxonomic controversy. Based on 76 *Euconulus* populations collected across Eurasia and North America, we empirically evaluated these competing taxonomic hypotheses through an integration of nDNA and mtDNA phylogenetics, shell morphometrics and various qualitative traits. Our results support the existence of five taxa: *Euconulus alderi* (Atlantic Europe to western North America), *E. fresti* sp. nov. (North America), *E. fulvus fulvus* (Europe), *E. fulvus egenus* (central Asia to Atlantic North America), and *E. polygyratus* (north-eastern North America). Each species-level entity possessed a unique suite of observable shell features allowing for accurate identification without need of DNA sequence information. Our data did not support the recent erection of *E. callopisticus*, *E. praticola*, and *E. trochiformis* to species-level status. This work also helps illustrate the importance of using consensus across DNA and shell/soft body features in assessing species-level taxonomy and in determining those features which allow for accurate identification. It furthermore documents the importance of basing taxonomic work on samples drawn from across the entire geographic and ecological range of the study group.


**Key words:** *Euconulus*, morphometrics, phylogenetics, species delimitation, taxonomy, terrestrial gastropod

**Introduction**
Land snails of the genus *Euconulus* Reinhardt, 1883 are frequent across the Holarctic both in modern (Cameron, Pokryszko, & Horsáková, 2010; Horsáková, 2006; Nekola, 2014) and fossil (Juríčková, Horsáková, Horáčková, Abraham, & Ložek, 2014; Ložek, 2001) assemblages. These species are characterized by their minute size and simple shell morphology, having conical, closely coiled shells of brown to amber colour, generally ranging from 2.3–3.5 mm in diameter (Kerney & Cameron, 1979; Pilsbry, 1946). The genus is considered native across the Holarctic (Pilsbry, 1946), and introduced in other parts of the world (Stanisic, Shea, Potter, & Griffiths, 2010). The taxonomy of the genus and its phylogenetic position have been subject to ongoing debate (Baker, 1963; Hausdorf, 1998; Hyman, Ho, & Jermiin, 2007), with over 20 *Euconulus* species being described according to the worldwide malacological literature and databases (e.g., Baker, 1963; Galli, 2016; Kerney & Cameron, 1979; MolluscaBase, 2019; Pilsbry, 1946). Our former research suggests that except for the south-eastern USA, the Holarctic representatives of the genus appear restricted to a monophyletic clade containing taxa closely allied with *E. fulvus* (Müller, 1774) (Horsáková, Nekola, & Horsáková, 2019), which we here-after call the *E. fulvus* group.

Throughout the 20th century three members of the *E. fulvus* group were generally believed to exist in Eurasia: upland *E. fulvus* and *E. fulvus alaskensis* (Pilsbry, 1899) (Kamchatka; Pilsbry, 1946), with pale bodies, light yellow shells, and dull lustre, and wetland *E. alderi* (Gray, 1840) with darker bodies and shinier, darker shells. This changed with publication of the Check-list of the Non-marine Molluscan Species of Northern, Atlantic and Central Europe (CLECOM; Falkner, Bank, & von Proschwitz, 2001; Falkner, Ripken, & Falkner, 2002) with *E. trochiformis* (Montagu, 1803) being considered a senior synonym of *E. alderi, E. praticola* (Reinhardt, 1883) being used for most continental *E. alderi* populations, and...
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E. callopisticus (Bourguignat, 1880) being considered a valid species-level entity. Although clear diagnostic traits were not given to support these concepts, the data presented in Falkner et al. (2001, 2002) suggest that E. praticola occurs across continental and oceanic Europe, E. trochiformis is intermediate between E. praticola and E. fulvus and presumably limited to Atlantic regions, and E. callopisticus possesses shells taller than E. praticola and occurs in the Mediterranean region. While this approach has been followed in some recent works (e.g., Gargominy, Prié, Bichain, Cucherat, & Fontaine, 2011; Groh & Weitmann, 2007), it has also been rejected by others (e.g., Cameron, 2003) in part because no effort was made to confirm that type E. callopisticus and E. trochiformis match the above concepts (Welter-Schultes, 2012). The use of E. praticola in preference to E. alderi, without acceptance of E. callopisticus and E. trochiformis, has also been adopted by some European authors (e.g., Čejka, Dvorák, Horsák, & Steffek, 2009; Horsák, Juříčková, & Picka, 2013; Körnig, Hartenauer, Unruh, Schnitter, & Stark, 2013; Zettler et al., 2006).

In North America this group was historically thought to represent only E. fulvus and E. f. alaskensis (Pilsbry, 1946). While the range of the former was believed to cover all but the south-eastern quarter of the continent, the latter was thought limited to the Western Cordillera. Subsequently two species were added. Based on comparison with European material Frest (1990) reported E. alderi from Iowa fens. This entity eventually was reported throughout the north-central/north-eastern USA (Nekola, 1998, 2002, 2004, 2008) and adjacent eastern Canada (Nekola, 2009) with populations from British Columbia and Ontario being considered E. praticola (Forsyth, 2004; Forsyth & Oldham, 2016). Additionally, while Euconulus polygyratus (Pilsbry, 1899) was originally described as a subspecies of the south-eastern USA E. chersinus (Say, 1821), and without empirical validation risen to species rank (Grimm, 1971), previous DNA sequence analysis confirmed its species-level status and showed it to be a member of the E. fulvus group (Horsákova et al., 2019).

The frequency of the E. fulvus group across the Holarctic combined with its contentious taxonomy suggests that a formal revision is warranted. We have previously taken an integrative approach that considers consensus among mtDNA and nDNA sequences, conchology, ecology, and biogeography to determine the number of biologically supported taxa (e.g., Pupilla – Nekola, Coles, & Horsák, 2015; Vertigo – Nekola et al., 2018). By applying this methodology to Euconulus, we can determine not only the actual number of empirically defensible taxonomic concepts but also provide robust information regarding their geographic and ecological range and identify those conchological features which provide accurate species-level assignments.

Our goal in this contribution is to present an empirically supported species-level taxonomy of the E. fulvus group in which we formally conduct the nomenclatural acts – including a description of a new species – suggested by, but not undertaken in, our previous work concerning the delimitation of cryptic species (Horsákova et al., 2019).

Materials and methods

Taxonomic coverage

Analysed material was primarily obtained from collections made by the authors from 2000–2018. These represent approximately 900 Euconulus populations from Eurasia (Michal Horsák collection) and 1000 from North America (Jeffrey Nekola collection). Specimen selection was grounded in the fact that taxonomic diagnoses within this genus have until now been limited to a small suite of conchological criteria with unknown utility. Using the most liberal assignment of species status for both Europe (Falkner et al., 2002) and North America (NatureServe, 2018) we selected material in order to cover known conchological and geographic variation of all putative taxa (E. alderi/trochiformis, E. callopisticus, E. fulvus, E. f. alaskensis, E. praticola, E. polygyratus) using the diagnostic traits indicated in Falkner et al. (2002), Frest (1990), Horsák et al. (2013), Pilsbry (1946), and Welter-Schultes (2012). We excluded a potentially distinct lineage from Japan due to the very limited number of observed populations in combination with their considerable observed genetic variation. Resolution of their status is left to future investigators.

In addition, we also analysed 12 E. callopisticus (and images of a syntype which could not be loaned) from the Bourguignat collection (Natural History Museum of Geneva, Switzerland; MHNG 103698, 103700 and 103701) and images of two E. trochiformis syntypes from the Montagu collection (Royal Albert Memorial Museum & Art Gallery, Exeter; EXEMS Moll4313; see Oliver & Morgenroth, 2018). Because it was not possible to use this material for DNA analyses (all were over a century old, were mostly empty, with any remaining dried tissue requiring either shell destruction or degradation to extract DNA – see Nekola et al., 2018) they were only used for morphological evaluation and morphometry.

DNA sequence and phylogenetic analysis

A total of 76 specimens were used in the phylogenetic analyses, representing the known geographic and ecological range of all currently recognized Eurasian and North American nomens including: 7 E. alderi/E.
trochiformis, 9 E. aff. alderi, 2 E. callopisticus, 29 E. fulvus, 3 E. f. alaskensis, 20 E. praticola, and 6 E. polygyratus (Fig. 1, Supplementary Material – Appendix S1). An individual of E. trochulus (Reinhardt, 1883) from Oklahoma, USA (representing the E. chersinus group sensu Pilsbry, 1946) was used for outgroup comparison.

DNA extraction, PCR amplification, and DNA sequencing was performed using standard protocols (see Horsáková et al., 2019). We analysed two mitochondrial [16S ribosomal RNA (16S) and cytochrome oxidase subunit II (COII)] and two nuclear [internal transcribed spacers 1 and 2 of the ribosomal RNA gene complex (ITS1 & ITS2)] amplicons using primers and thermal profiles listed in Table 1. Geneious v. 8.0.2 (Biomatter Ltd) was used for assembling forward and reverse strands into one sequence which was manually checked for potential misreads. The protein-coding COII fragment was translated into amino acids to check for the presence of erroneous stop codons. GenBank accession numbers of all sequences used in the phylogenetic analyses are available in Appendix S1.

Table 1. Forward (F) and reverse (R) primer sequences used for PCR with anneal temperatures and primer design authors.

<table>
<thead>
<tr>
<th>Region</th>
<th>Sequence</th>
<th>Anneal</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>COII (F)</td>
<td>5'-AAATAATGCTATTTTCATGAYCAYGC-3'</td>
<td>45°C</td>
<td>Hugall, Moritz, Moussalli, &amp; Stanisic (2002)</td>
</tr>
<tr>
<td>COII (R)</td>
<td>5'-GCTCCGCAAATCTCTGARCATG-3'</td>
<td>45°C</td>
<td>Hugall et al. (2002)</td>
</tr>
<tr>
<td>16S (F)</td>
<td>5'-GCGCTGGTTATCAAAACAT-3'</td>
<td>52°C</td>
<td>Palumbi et al. (2002)</td>
</tr>
<tr>
<td>16S (R)</td>
<td>5'-CCGTTYGAATCAGATCAYGT-3'</td>
<td>52°C</td>
<td>Palumbi et al. (2002)</td>
</tr>
<tr>
<td>ITS1 (F)</td>
<td>5'-TAACAGTTTTCCGTATGGA-3'</td>
<td>52°C</td>
<td>Armbruster &amp; Bernhard (2008)</td>
</tr>
<tr>
<td>ITS1 (R)</td>
<td>5'-TCATATAATTTCGCAGCTAG-3'</td>
<td>52°C</td>
<td>Nekola et al. (2018)</td>
</tr>
<tr>
<td>ITS2 (F)</td>
<td>5'-CTAGCTGAGAATATGGA-3'</td>
<td>52°C</td>
<td>Wade &amp; Mordan (2000)</td>
</tr>
<tr>
<td>ITS2 (R)</td>
<td>5'-GTTTCACGTACTCTTGAA-3'</td>
<td>52°C</td>
<td>Nekola et al. (2018)</td>
</tr>
</tbody>
</table>

Multiple sequence alignments were performed in MEGA v. 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) using ClustalX with the default parameters of the IUB weight matrix as implemented, and checked by eye for potential errors. The two mitochondrial (mtDNA) amplicons were concatenated into a single construct as were the two nuclear (nDNA) fragments. Four different phylogenetic reconstruction methods, each employing very different analytical assumptions, were then separately run on the mtDNA and nDNA constructs to identify well-supported, shared structures. Mega v6.0 was used to conduct Neighbour-joining (NJ), using maximum composite distance including transitions and transversions with pairwise gap deletion. Maximum parsimony (MP) was conducted in TNT (Goloboff, Farris, & Nixon, 2008) using traditional search with 1000 replicates of Wagner trees, one random seed, Tree Bisection and Reconnection branch-swapping algorithm and 10 trees to save per replication. ML analysis was conducted using RAxML v 8.2 (Stamatakis, 2014) while BI analysis was performed using MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001). Both the mtDNA and nDNA constructs were first partitioned into two genes each with optimum base pair substitution models being identified using jModelTest v. 2.1.10 (Darriba, Taboada, Doallo, & Posada, 2012). Internal node support was assessed via 1000 non-parametric bootstrap replicates (Felsenstein, 1985). Trees were visualized using FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Full methodology is reported in Horsáková et al. (2019).

We also conducted preliminary analyses on 10 Euconulus mtDNA cytochrome oxidase I (COI) sequences from France, kindly provided by Olivier Gargominy (personal communication, 19 December 2018), together with 29 COI from specimens we collected across Eurasia (data available upon request).
Shell morphometrics

Shell measurement protocols follow Horsáková et al. (2019). A total of 106 shells from 33 populations in the Czech Republic, Slovakia, Poland, Switzerland, Bulgaria, Norway, Sweden, Russia, USA, and Canada were analysed (Appendix S2), along with two syntype E. trochiformis and three E. callopisticus (see above). Photo-micrograph images of each shell were generated using an Olympus SZX7 with Olympus C-7070 Wide Zoom camera and QuickPHOTO MICRO software. All shells were measured by the authors except for the E. trochiformis syntypes which were imaged and measured by P. Graham Oliver at the National Museum Wales (Cardiff) using identical protocols.

Because of the lack of determinant growth and absence of well-defined conchological structures to demarcate adult individuals in Euconulus, we only measured shells of at least four whorls and then compiled ratios between seven conchological traits, with the number of whorls being counted following Cameron (2003). These ratios were then subjected to discriminant correspondence analysis (see Horsáková et al., 2019). Basic descriptive statistics of all directly measured traits and calculated ratios are shown in Appendix S2, and an illustration of shell measurements is shown in Appendix S3. All calculations were conducted in R version 3.5.2 (https://www.r-project.org/), using the ‘ade4’ (Dray & Dufour, 2007), and ‘vegan’ (Oksanen et al., 2017) packages.

We complemented these analyses by visually evaluating eight additional qualitative morphological characters from each individual, including: body tissue and mantle colouration, presence of a keel, sillon (‘furrow’ in French, used by Welter-Schultes (2012) to demarcate the spiral grooves inset into the bottom shell surface) strength, shell colour and lustre, protoconch and teleoconch microsculpture. The latter were documented using a digital Keyence VHX-5000 microscope with ZS-20 and ZS-200 objective lenses.

Species delimitation

We choose to demarcate a species as being distinct once it has become a largely independent evolutionary unit (Horsáková et al., 2019). We do not use any of the various sequence-based species delimitation methods as these may provide inaccurate results especially for groups with recent diversification histories (e.g., Wei et al., 2016), and require the making of questionable assumptions regarding evolutionary rate (e.g., Nekola, Coles, & Berghthorsson, 2009). Instead, we first searched the mtDNA and nDNA trees for jointly held, highly supported, reciprocally monophyletic groups containing the same individuals. The conchological, geographic, and ecological ranges for each were then subjected to morphometric and qualitative evaluation. Such groups are considered to represent valid species-level entities when unique patterns occur across a consensus of data. A priori species identifications were then updated based on these integrative findings.

Results

Phylogenetic reconstruction

DNA sequence data were obtained for 75 specimens in the mitochondrial and 76 specimens in the nuclear construct (Supplementary Material – Appendix S1). Amplicon lengths were 379–381 bp for 16S, 502 for COII, 652–664 for ITS1, and 862–890 for ITS2. Twenty-five variable base pair positions were identified in 16S, 109 in COII, 22 in ITS1, and 21 in ITS2. Because NJ, MP, ML, and BI all generated essentially the same topologies we used only ML to illustrate phylogeny (Fig. 2). Support values were generally higher in the mtDNA dataset presumably due to greater base pair variability.

Four reciprocally monophyletic clades showing high support in both the mtDNA and nDNA trees were observed: (1) Eurasian E. alderi/trochiformis, E. callopisticus, E. pratricoli, and far western North American E. aff. alderi; (2) North American E. aff. alderi east of the Cascade/Sierra Nevada Mountains; (3) E. polygyrus; and (4) European E. fulvus. The remaining Asian and North American E. fulvus fell into multiple weakly defined, somewhat geographically structured clades, which we choose to interpret as representing a single poorly defined subspecies due to their uniformly very low support, inconsistency of placement between nDNA and mtDNA clades, lack of identifiable diagnostic macro-scale features unique to individual clades, and evidence for hybridization (for full reasoning see Horsáková et al., 2019; and Discussion in the present study). Within-group variability ranged from 3.2–13.5 bases in COII + 16S and 0.3–2.6 bases in ITS1 + ITS2. Between group variability ranged from 23.7–32.2 bases in COII + 16S and 2.8–6.8 bases in ITS1 + ITS2 (Supplementary Material – Appendix S4).

Material initially identified as E. alderi/trochiformis was polyphyletic, with western/Atlantic populations being strongly associated with E. callopisticus and E. pratricoli and alpine populations being strongly associated with E. fulvus. This pattern was replicated in preliminary reconstructions limited to COI sequence from eastern France and Eurasia (results available upon request). Additionally, material initially identified as North American E. aff. alderi was polyphyletic, with most populations representing a highly supported
Figure 2. Maximum likelihood tree for the *Euconulus fulvus* group based on mtDNA (16S + COII) (left) and nDNA (ITS1 + ITS2) (right). Support values of >70% for NJ (upper left, bold font), MP (upper right, italic font), ML (lower left, normal font), and in posterior probabilities of >95% for BI (lower right, bold italic font) are shown next to the corresponding nodes. Only highly supported nodes in at least one of the methods are labelled. Colours represent individual source: blue – Europe, green – Asia, red – North America.
distinct clade. However, an eastern taiga specimen was strongly associated with non-European *E. fulvus*, and far western/arctic specimens were strongly associated with *E. alderi/trochiformis*.

**Morphometric comparison**

Single quantitative shell measurements demonstrated considerable overlap and were generally ineffective in distinguishing groups (Table 2a). However, multivariate ordination of measured ratios did illustrate partitioning (Fig. 3.1). The principal axis of variation was most closely associated to the ratio between shell size (height or width) and the number of whorls (e.g., coiling tightness/expansion rate; $r^2 = 0.967$), with *E. polygyrus* possessing much tighter coils and reduced coil expansion (Table 3a). As a result, individuals of the same diameter possess 1–2 more whorls than other group members. This species was so distinct that it forced the remaining groups to overlap in a non-informative way. Discriminant analysis was thus repeated following removal of *E. polygyrus*. In this new analysis (Table 3b, Fig. 3.2) the first axis was again most correlated with coiling ratio ($r^2 = 0.918$). For both analyses the second axis was poorly explained by any single trait, and represented a complex combination of shell height, width, and aperture measurements. While it was most correlated in the full analysis with aperture height to body whorl height ratio and in the reduced analysis to shell height to width ratio, these factors explained little observed variation ($r^2 = 0.179$ and $r^2 = 0.217$, respectively). For this reason the second axis is best seen as not reflecting any particular calculated shell ratio.

In cases of morphometric overlap qualitative morphological features were found to clearly separate groups (Table 2b). In comparison with *E. fulvus*, European *E. alderi/trochiformis* and North American *E. aff. alderi* have darker red-brown shells with a more shiny lustre, reduced teleoconch microsculpture, stronger sills, and a darker body. Eurasian/western North American *E. alderi/trochiformis* differ from North American *E. aff. alderi* in possession of a uniform dark mantle, rounded margin, stronger protoconch microsculptural ribs and weak to absent teleoconch microsculpture. North American *E. aff. alderi* is characterized by a mottled mantle, prominent keel, weak to absent protoconch sculpture, and more prominent microsculpture (especially spiral lines) on the teleoconch. No features allowed for definitive separation of European and non-European *E. fulvus*, with all trait characters being observed across all groups. However, European *E. fulvus* tended to have a tighter coiling ratio with more constant rate of whorl expansion with uniform grey mantle.
tissue, while non-European *E. fulvus* tended to possess more lax coiling with an initial low and then more rapid rate of whorl expansion and mottled mantle tissue.

**Systematic treatments**

Based on these results we choose to recognize four species and one subspecies within the *E. fulvus* group.
Their valid names are: *E. alderi*, *E. fresti* sp. nov., *E. fulvus*, *E. fulvus egenus*, and *E. polygyratus*. To assist identification, results have been summarized via an illustrated taxonomic key (Fig. 4).

**Family Euconulidae** H.B. Baker, 1928  
**Genus Euconulus** Reinhardt, 1883

*Euconulus* Reinhardt, 1883: 86.

**Type species.** *Helix fulva* Müller, 1774.

*Euconulus alderi* (Gray, 1840)  

**Type locality.** Great Britain

**Synonyms.** *Hyalina praticola* Reinhardt, 1883: 40, type locality: Germany – Brandenburg.

**Material examined.** Twenty-two populations from Europe (Austria, Bulgaria, Czech Republic, France, Poland, Romania, Slovakia, Sweden, Switzerland, and United Kingdom), Asia (Russia: Western Siberia) and North America (USA: Alaska and California); for details see Supplementary Material – Appendix S1.

**Phylogenetics.** *Euconulus alderi* exists as a monophyletic clade across all reconstruction methods in both the nDNA and mtDNA constructs (Fig. 2). Preliminary analysis of COI from France and other Eurasian populations also supported the presence of only *E. alderi* and *E. fulvus fulvus*. Our data were unable to establish with high support the most closely related sister taxon to *E. alderi*.

**Conchology.** This species is characterized by dark, cinnamon-brown shell with a shiny surface, strongly reduced teleoconch microsculpture, protoconch microsculpture limited to rounded, widely spaced radial ribs, and a dark animal with uniform mantle colour (Figs 4 [v.a–c], 5.1). It typically possesses strong sills (Table 2, Figs 4 [v.d], 7.2). *Euconulus fulvus* differs in possessing a yellow, duller shell with sharper and denser microsculptural ribs on both the teleoconch and protoconch and a grey animal body. While European *E. fulvus fulvus* sills are typically weaker, considerable overlap is observed with *E. fulvus egenus*. *Euconulus alderi* differs from the similar North American *E. fresti* sp. nov. in possessing a greater coil expansion rate, rounded margin, stronger protoconch microsculpture, weaker teleoconch microsculpture, and possession of uniformly coloured mantle tissue.

**Ecology.** *Euconulus alderi* is restricted to base-rich wetlands, ranging from open fens to wet riparian and other swampy forests.
Biogeography. Atlantic Europe east across the Eurasian and Alaskan taiga and tundra to the west shore of Hudson’s Bay in the North American arctic and south along the Western Cordillera to the southern Sierra Nevada in California.

Nomenclatural notes. Falkner et al. (2002) erect species from the Atlantic/Alpine (E. trochiformis), continental Europe (E. praticola), and eastern France (E. callopisticus) to replace E. alderi; however, none were empirically supported by either genetic or conchological data. While British material referable to the Falkner et al. (2002) concept of E. trochiformis possesses DNA sequence solidly rooted in the highly supported E. alderi clade, material corresponding to E. trochiformis in the sense of Falkner et al. (2002) from high altitude alpine habitats is actually E. fulvus. Furthermore, even if genetic partitioning had existed, nomens were incorrectly applied because type material for both E. callopisticus and E. trochiformis actually represents E. fulvus. As a result, the earliest available nomen representing this entity is E. alderi, with the original description stating: “Mr. Alder observes that … it is darker coloured and with very delicate and beautiful concentric striae on the base, only visible with a high magnifier, which induced him at first to consider it distinct …” (Turton & Gray, 1840).

Euconulus fresti sp. nov.
Figs 3, 6, 7.1

GenBank accessions: MK266537; MK266543-6; MK299610; MK299616-9; MK299700; MK299707-10; MK299793; MK299800-3; MN706207; MN706200-3.

Diagnosis. Glossy dark cinnamon-brown shell, strong sillons, overall appearance similar to E. alderi but with tighter coiling, mottled mantle tissue, keeled margin on shells of all ages, weak to absent protoconch microsculpture and distinct teleoconch microsculpture of spiral lines with weak ribs. Within the E. fulvus group, this species uniquely possesses 350 T in the aligned ITS2 sequence (Supplementary Material – Appendix S5).

Description. Shell height 1.9–2.1 mm, width 2.5–2.7 mm (holotype 1.9 × 2.6 mm); width vs. number of whorls 0.52–0.61 (holotype 0.54), height to width ratio 0.7–0.8 (holotype 0.75). Shell dark red-brown with glossy lustre, squat-conical shape, moderate suture, aperture crescent-shaped with a simple edge, no umbilicus, 4.8–5.0 whorls in fully adult individuals (holotype 4.75), relatively tightly coiled with whorl width increasing at a constant 1.5–1.8 × rate per rotation, keeled periphery present even in fully adult individuals (Fig. 4 [iv.b]). Protoconch microsculpture reduced to absent, limited (when present) to very weak spiral lines (Figs 6 [i], 7.1); teleoconch microsculpture of distinct spiral lines with weaker thread-like ribs (Fig. 6 [ii–iii]). Sillons strong and sparse (Figs 4 [iv.c], 6 [iv], 7.1, 4 [iv.c]). Animal body almost black with a dark-grey mottled mantle (Fig. 4 [iv.a]).

Figure 5. Live individuals of Euconulus alderi (1), E. fulvus fulvus (2–3) and E. fulvus egenus (4): 1, Slovakia; 2, Crimea; 3, Northern Norway; 4, Western Siberia, Russia. Photo: R. Coufal (1, 3), S. Leonov (2), M. Horsáková et al.
Holotype (Figs 6.1, 6 [i and iv], 7.1). ANSP 478687, Martelle Fen, Jones County, Iowa USA: sedge mat in pasture (42°27′N, 91°19′36″W); 27 October 2002, J.C. Nekola.


Other examined non-Type material. NMW.Z.2005.011.00781, NMW.Z.2005.011.01547 representing approximately 200 shells from Minnesota and Maine, USA. Also 208 lots representing 6320 individuals from Alberta, British Columbia, Iowa, Maine, Manitoba, Massachusetts, Michigan, Minnesota, Nevada, New Hampshire, New Mexico, New York, Ontario, Utah, Vermont, and Wisconsin in the Nekola collection.

Etymology. The specific epithet honours Terrence J. Frest (Supplementary Material – Appendix S6), the first researcher to recognize the existence of this species. Terry was a tireless field researcher and one of the most ardent champions of North American terrestrial gastropod conservation during the latter decades of the 20th century.

Phylogenetics. This species exists as a highly to very-highly supported monophyletic clade across all reconstruction methods in both the nDNA and mtDNA constructs (Fig. 2). While the phylogenetic reconstructions based on mtDNA suggest a sister-group relationship with *E. polygyratus*, the data were insufficient to resolve the relationship with high support (observed values <50). The potential validity of this sister relationship, however, is suggested by shared...
reduced protoconch microsculpture and higher coiling ratio as compared to *E. alderi* and *E. fulvus*.

Comparisons with other species. *Euconulus fresti* sp. nov. is consistently differentiated from *E. alderi* based on its weaker protoconch microsculpture, stronger teleoconch microsculpture, keeled margin, and mottled mantle (Figs 6, 7, Table 2). *Euconulus polygyratus* differs in its dull yellowish shell, unmarked shell bottom, narrower whorls, and pale animal body. While *E. fresti* sp. nov. typically has tighter coiling than *E. fulvus egenus*, it can be difficult to distinguish in some wetland and cool, humid forest *E. fulvus egenus* populations that possess reduced microsculpture, shinier lustre, tighter than normal coiling ratio, and keeled margins. However animal colour in these individuals remains grey, their shells remain yellowish, with microsculptural ribs on the protoconch always being stronger than any spiral lines.

**Ecology.** Very similar to *E. alderi*, being particularly common in open sedge mats of fens, rich conifer/ash swamps, sedge meadows, and shrub carrs. It is most likely to co-occur with *E. fulvus egenus* in the latter habitat.

**Nomenclatural notes.** This species has previously been reported as *E. alderi* from the USA and eastern Canada (Frest, 1990; Nekola, 1998, 2002, 2003, 2004, 2008, 2009, 2012, 2014) and from British Columbia and Ontario as *E. praticola* (Forsyth, 2004; Forsyth & Oldham, 2016). In our earlier work (Horsáková et al., 2019) it was referred to as *E. aff. alderi*.

Euconulus fulvus fulvus (Müller, 1774)

*Helix fulva* Müller, 1774: 56.

**Type locality.** Denmark – Frederiksdal near København.


**Material examined.** Nineteen populations from Europe (Austria, Czech Republic, Iceland, Norway, Poland, Romania, Slovakia, Sweden, and Switzerland), Asia (Russia: Western Siberia) and North America (USA: Alaska and California); for details see Supplementary Material – Appendix S1. In addition, 12 shells of *E. callopisticus* (and images of a syntype which could not have been loaned) from the Bourguignat collection (MHNG 103698, 103700, and 103701) and two syntype
shells of *E. trochiformis* from the Montagu collection (EXEMS Moll4313) were also examined and found to represent *E. fulvus fulvus*. With respect to *E. callopisticus* we also thoroughly revised our own southern European material (46 lots in the Horsák collection from France, Switzerland, and Italy).

**Phylogenetics.** *Euconulus fulvus fulvus* exists as a monophyletic clade across all reconstruction methods in both the nDNA and mtDNA constructs (Fig. 2), with an exception of one ambiguously assigned specimen in the nDNA tree (E63). Such anomalous sequence in the nuclear rRNA gene complex has been found to be taxonomically uninformative in other pulmonate land snail groups (Nekola et al., 2018). The placement of Bohemian and Swedish specimens outside of this clade in nDNA tree is likely due to hybridization with *E. fulvus egenus* (perhaps during the Pleistocene; see Horsák et al., 2019). The data were not sufficient to resolve with high support the phylogenetic relationships of *E. fulvus fulvus* with other species in the group. The monophyletic nature of *E. fulvus fulvus* was also supported in preliminary analysis of COI sequence from eastern France and across Eurasia.

**Conchology.** This taxon is most easily separated from *E. alderi* and *E. fresti* sp. nov. by its typically lighter shells with stronger protoconch and teleoconch microsculpture, resultant duller lustre, weaker sillon, and grey animal body (Table 2b, Figs 4 [ii.a–d], 5.2, 7.3). While no definitive traits separate all *E. fulvus fulvus* from *E. fulvus egenus*, *E. fulvus fulvus* individuals tend to possess a tighter coiling ratio, more constant whorl expansion, weaker sillon, and a uniformly coloured mantle. However, some European *E. fulvus fulvus* possess laxer coiling, discontinuous whorl expansion, strong sillon, and mottled mantle tissue (Fig. 5.3). Considerable variability is also noted in spire height and other conchological traits. This plasticity appears at least partially responsible for erection of *E. callopisticus* and *E. trochiformis* (Figs 3, 9). It should also be mentioned that ecophenotypic variability is substantial with populations from high-elevation and high-latitude habitats developing a darker shell with less prominent teleoconch microsculpture and dark-grey mottled mantle tissue (Figs 4 [ii.b], 5.3, 9.viii). Because these populations appear intermediate between *E. alderi* and *E. fulvus fulvus*, they correspond to the concept of *E. trochiformis* in Falkner et al. (2002). This led to our initial misidentification of these individuals and polyphyleticism for *E. trochiformis* in the phylogenetic trees.

**Ecology.** This species occurs in a wide variety of upland forest, shrubland, heath, and grassland habitats across the soil acidity gradient. It is one of the more acid-tolerant species in the European fauna. It also colonizes acid wetland sites, especially at high elevations and latitudes.

**Biogeography.** This species ranges across western Eurasia from Iceland and Atlantic Europe to just east of the Urals.

**Nomenclatural notes.** Examination of *E. callopisticus* and *E. trochiformis* types (Figs 3, 9) documents that both are junior synonyms of *E. fulvus fulvus* and should not be associated with *E. alderi* and its synonyms.

**Euconulus fulvus egenus** (Say, 1825)

*Helix egena* Say, 1825: 120.
Type locality. USA – Bank of the Delaware River about 10 miles from Philadelphia.


Material examined. Twenty-three populations from Asia (Russia: Altai, Yakutia, and Western Siberia) and North America (Canada: Québec, British Columbia, and Labrador; USA: Alaska, California, Idaho, Maine, Minnesota, New Mexico, Texas, and Utah); for details see Supplementary Material – Appendix S1.

Phylogenetics. This taxon is represented by multiple weakly defined, somewhat geographically structured clades, covering central Asia across Beringia to North America (Fig. 2). As explained in detail in Horsák et al. (2019), these cannot be considered biologically valid species because of their uniformly very low support, inconsistent specimen assignment between the nDNA and mtDNA clades, and lack of any diagnostic macro-scale features. Since evidence also exists of hybridization between central Asian populations and E. fulvus fulvus (see Horsák et al., 2019), we consider only a single species to be present, with the non-European populations being designated as a subspecies.

Conchology. While no unique conchological features demarcate E. fulvus egenus, it tends to differ from E. fulvus fulvus by possessing more lax coiling, a more discontinuous whorl expansion rate (being initially slow and then rapidly increasing around the second whorl), stronger sillons, more frequent presence of a keel, and possession of strongly mottled mantle tissue (Table 2b, Figs 4 [iii.a–c], 5.4, 7.4). However individuals demonstrating these traits can be found across the entire species range. Individuals sourced from wet taiga and other humid, cool habitats (such as algific talus slopes in the USA upper Midwest) sometimes resemble E. fresti sp. nov. in terms of tighter coiling, reduced shell microsculpture, and shinier lustre. However these always maintain a lighter shell colour, grey animal body, and always have the microsculptural ribs being stronger than any spiral lines. Shells from the central Asian clade often possess the tighter coiling ratio of E. fulvus fulvus but the mottled mantle of E. fulvus egenus.

Ecology. One of the most catholic upland snails, being found from forests to grasslands across the entire soil moisture and acidity gradient.

Biogeography. Ranges from east of the Urals in central Asia to the Atlantic coast in the north-eastern USA and Canada, north into the arctic tundra and south into Mexico along the Rocky Mountains.

Figure 9. Shell variation of European Euconulus fulvus and its junior synonyms: 1, E. trochiformis, syntype shell from the series no. EXEMS Moll4313: i, 2.61 × 3.35 mm (height × width); ii, teleoconch top microstructures in SEM; 2, E. callopisticus, original collections: iii, MHNG-MOLL-103700, 1.98 × 2.72 mm; iv, teleoconch top microstructures of the shell from MHNG-MOLL-103701 and v, the same collection, 2.26 × 2.87 mm; vi, MHNG-MOLL-103700; 3, E. fulvus: vii, mountain spruce forest, Moravia, Czech Republic, 2.30 × 3.09 mm; viii, mountain alkaline fen, Passo della Nuovena, Switzerland, 1.93 × 2.81 mm; ix, mountain alkaline fen, Zermatt, Switzerland; x, mountain spruce forest, Moravia, Czech Republic.
Nomenclatural notes. We have chosen *egenus* as the name for this entity because it appears to represent the earliest *nomen* applied to any non-European *E. fulvus* population. Note that the specific epithet was modified to *'egenus'* to accommodate the gender shift of the generic epithet from *Helix* to *Euconulus*. Although material identified as *Helix egena* by Say no longer exists at ANSP, given the original description (‘It is much broader than the *H. chersina*’) it can represent nothing else: In this part of Pennsylvania only three *Euconulus* can potentially exist (Hubricht, 1985) – *E. fulvus*, *E. fresti* sp. nov., and *E. polygyratus*. Prior to Grimm (1971) *E. polygyratus* was considered a form of *E. chersinus*. As shown above, *E. fulvus egenus* possesses much broader shells and whorls than either of these species.

*Euconulus polygyratus* (Pilsbry, 1899)

*Conulus chersinus polygyratus* Pilsbry, 1899: 116.

Type locality. Grand Rapids, Michigan.

Material examined. 6 populations from North America (Canada: Ontario; USA: Iowa, Maine, Michigan, Ohio, and Virginia); for details see Supplementary Material – Appendix S1.

Phylogenetics. This species exists as a monophyletic clade across all reconstruction methods in both the nDNA and mtDNA constructs (Fig. 2). All phylogenetic reconstructions based on the mtDNA construct suggest with very low support (values <50) a sister-group relationship with *E. fresti* sp. nov.

Conchology. Elevated to a full species by Grimm (1971), its shells are highly distinct from any other member of the *E. fulvus* group by their very slow whorl expansion rate allowing for 1–2 additional whorls to exist for a given diameter (Figs 3, 4 [i.a]). It is also distinguished by its strongly lunate aperture, domed spire (Fig. 4 [i.b]), reduced microsculpture, and absence of sills (Table 2b).

Ecology. This species is characteristic of rich upland, often rocky forest.

Biogeography. Ranges from the Atlantic Provinces of Canada west through the Great Lakes to north-central Minnesota and south to north-eastern Iowa, northern Illinois, the Ohio River valley and Maryland. It extends south along the crest of the Appalachians to western North Carolina.

Discussion

Global diversity of *Euconulus*

The review of literature sources and data in online databases (Baker, 1963; Falkner et al., 2002; Galli, 2016; Kerney & Cameron, 1979; MolluscaBase, 2019; Pilsbry, 1946; Poppe & Poppe, 1994–2019) revealed approximately 20–30 extant *Euconulus* species worldwide, with some being considered endemic, e.g., to Japan (Clench & Turner, 1962), Hawaii (Johnson, 1996), and Bermuda Islands (Bieler & Slapcinsky, 2000). However, an actual species diversity of this genus is very difficult to estimate mainly for two reasons. First, taxonomists often tended to oversplit these continental-range species based on their local forms, such as seen for *E. callopisticus* and *E. trochiformis* in our study. Second, the delimitation of the genus itself is problematic due to its simple shell morphology, making it similar to other genera, mainly *Kaliella* and *Parakaliella*. Thus, some of the putative *Euconulus* species might in fact belong to these related genera and vice versa (see Baker, 1963). For instance in Japan, the species *Parakaliella harimensis* (Pilsbry, 1901) remarkably resembles *E. fulvus* in all superficial morphological features (K. Kimura, personal communication, 19 January 2019; Kawase, Nishio, & Ichihara, 2013; Kawase & Ichihara, 2018). To resolve all these taxonomic issues will require a comprehensive phylogenetic analysis including all of the putative taxa related to *Euconulus*. Our discussion is therefore limited to the Holarctic species within the *E. fulvus* group, which are now well phylogenetically supported and taxonomically clarified.

Our data clearly show that only two *Euconulus* species are present in Europe, *E. alderi* and *E. fulvus*, with both *E. callopisticus* and *E. trochiformis* simply representing junior synonyms of *E. fulvus*, and *E. praticola* being a junior synonym of *E. alderi*. A potential occurrence of other species in Europe, including a putative undescribed Corsican endemic (Falkner et al., 2002), seems very unlikely, given the high and potentially confusing phenotypic plasticity of *E. fulvus* along with the typically large ranges expressed by *Euconulus* species.

The erection of these junior synonyms was probably related to an overemphasis on some morphological traits in isolation along with poor understanding of intraspecific plasticity within the genus. Such conchologically-based over-splitting is not unique to *Euconulus* but is rather common among shelled terrestrial gastropods in general (Elejalde, Munoz, Arrebola, & Gomez-Moliner, 2005; Emberton, 1995; Köhler & Burghardt, 2016).

The extra-tropical Asian fauna also includes only two species, *E. alderi* and *E. fulvus*, with both subspecies of
the latter, *E. fulvus fulvus* and *E. fulvus egenus*, being present in Western Siberia). *Euconulus fulvus* has also been reported as an exotic from Australia (Stanisic et al., 2010), New Zealand (Climo, 1979), and South America (e.g., Maceira, Miquel, Espinosa, Virgillito, & Lauranzon, 2013; Silva, 2007). Our identification of a genetically distinct lineage within the *E. fulvus* group in Japan (Horsáková et al., 2019) indicates that a thorough revision of the entire group within East Asia is necessary once additional material has been secured.

In North America four species occur (*E. alderi, E. fulvus egenus, E. fresti* sp. nov., and *E. polygyratus*) with the latter two being endemic to the continent. Besides these, the *E. chersinus* group – consisting of multiple additional genetically validated, yet taxonomically still unresolved species (Horsáková et al., 2019) – is also endemic to south-eastern North America. Thus as also seen in pupillid microsnails (Nekola et al., 2018), the North American *Euconulus* fauna is over three times as species-rich as the European. However, across all terrestrial gastropods the European fauna alone is at least three times as rich as the North American (Nekola, 2014; Welter-Schultes, 2012). Why some microsnails are more diversified in the otherwise more depauperate terrestrial gastropods the European fauna is at least three times as species-rich as the European. However, across all terrestrial gastropods the European fauna alone is at least three times as rich as the North American (Nekola, 2014; Welter-Schultes, 2012). Why some microsnails are more diversified in the otherwise more depauperate North American fauna remains undetermined, with the answer likely shedding light on important macro-evolutionary processes.

**Shell trait variation**

We note considerable variation within and overlap between taxa in quantitative shell traits such as number of whorls, shell height and width. Ratios of shell parameters tend to be more stable and species-specific, but still they only allow reliable identification of *E. polygyratus*. The most reasonable approach towards conchometry is therefore to analyse a suite of traits (or specifically, their ratios) in a multidimensional space, e.g., using a discriminant analysis (Horsáková & Meng, 2018; Horsáková et al., 2019). Remarkable levels of variation can also be seen for qualitative traits such as shell and mantle colouration. For instance, cold-climate, wetland populations of *E. fulvus fulvus* and *E. fulvus egenus* often exhibit slightly darker and glossier shells with somewhat darker mantle pigmentation.

Because it is frequent for an individual shell from one taxon to express a single trait more characteristic of another, we recommend basing identification on a consensus across multiple quantitative and qualitative factors. For the *E. fulvus* group this includes not only the ratios between shell height, width, body whorl height and aperture height and width and the number of whorls but also qualitative traits such as protoconch and teleoconch microsculpture, shell colour and lustre, sillon strength, animal body and mantle colour. A single shell might not be sufficient for a reliable species assignment, because occurrence of atypical specimens or aberrant forms is common (Cameron, 2016). Optimally, multiple representatives of the same population should be examined to find a consensus across morphological traits. In disputable cases, identification should always be verified with molecular data.

A similar use of shell trait consensus for robust species-level diagnoses has been previously applied to the micro-snail genera *Pupilla* and *Vertigo* (Nekola et al., 2015; 2018). Comparison of our findings across these genera illustrates two important points: (1) Traits historically used for identification do not necessarily allow for accurate species diagnoses. This can be seen in the use of raw whorl number in *Euconulus* and use of apertural lamellae architecture across *Pupilla* (Nekola et al., 2015) and in *Vertigo arthuri* Martens, 1882 shell forms (Nekola et al., 2018). (2) The utility of a given suite of traits to provide accurate species-level diagnoses appears to be group-specific. Thus, while animal and shell colour appears vital for assigning *Euconulus* species, these traits are of limited use in *Vertigo* (Nekola et al., 2018). Empirical documentation of those character suites which allow for accurate classification within a given group is thus a prerequisite for biodiversity assessment.

**Robust taxonomic assignment**

The present study is an example of a vital role of DNA data in land snail taxonomy. Without genetic information, we would not be able to determine the taxonomic utility of given traits, and distinguish those that are highly plastic from those taxonomically informative. While it is generally recommended to use multiple DNA markers for a robust species-level classification (e.g., Horsáková et al., 2019; Razkin et al., 2016; Sauer & Hausdorf, 2012), we consider it necessary to complement the initial molecular analyses with *post hoc* analysis of macro-scale traits to identify features useful for species-level diagnoses; this approach is applicable not only in land snails, but most other taxonomic groups.

Our data also caution against drawing taxonomic conclusions from datasets of limited taxonomic and geographic extent. If sampling had been conducted only on European *E. alderi* vs. *E. fulvus*, the presence of analogue *E. fresti* sp. nov. from North America would never have been observed. The complex nature of *E. fulvus* sp. lat. would also have been unobservable without sampling multiple populations across Eurasia and North America. In fact had analysis been limited to Europe and eastern North America, two distinct
species-level taxa would have been supported via DNA sequence and conchology. The evaluation of intermediate populations across Beringia and central Asia, however, revealed introgression in conchology and presumable presence of hybridization (Horskáková et al., 2019). This pointed us towards the decision to accept the traditional concept of E. fulvus as one species with a Holarctic distribution, despite its polyphyly in the DNA reconstructions. However, we consider this a tentative assignment that might – and should – be subjected to further scrutiny, perhaps using large-scale genome-wide data (e.g., RADseq technology). This approach has already proven useful in resolving phylogenetic relationships and species delimitation in the minute land snail genus Pyramidula (Razkin et al., 2016; Razkin, Gómez-Moliner, Vardinoyannis, Martínez-Ortí, & Madeira, 2017).

Taxonomic summary

Four species and one subspecies can be distinguished within the Holarctic Euconulus fulvus group; three are present in Eurasia (E. alderi, E. fulvus fulvus, E. fulvus egenus) while four are found in North America (E. alderi, E. fresti sp. nov., E. fulvus egenus, E. polygyratus). Euconulus fresti sp. nov. and E. polygyratus are endemic to North America; E. fulvus fulvus to western Eurasia. Euconulus trochiformis and E. callopisticus represent junior synonyms of E. fulvus, while E. praticola represents a junior synonym of E. alderi. North American E. fresti sp. nov. resembles E. alderi in both morphology (dark body and glossy shell) as well as ecology (wetland-restricted), but differs in a suite of diagnostic shell and body features. Euconulus polygyratus represents a valid species within the E. fulvus group. Earlier taxonomic over-splitting is likely linked to an overemphasis of single shell traits, as well as poor understanding of intraspecific conchological plasticity within this genus.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here: https://dx.doi.org/10.1080/14772000.2020.1725172.

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References


Integrative taxonomic consideration of the Holarctic Euconulus fulvus


**Supplemental material**

**Appendix S1.** Taxon name, *a priori* identification (prior to revision), location, habitat information, sample code and GenBank accession number for each of the analysed *Euconulus* specimens.

<table>
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<th>State / Province / region</th>
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*Euconulus fresti* sp. nov.

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**Euconulus fulvus fulvus**

Czech Rep. | fulvus | Krušně hory Mts. | Seepage in Ash forest | 50.6673 | 13.6708 | E80 MK266516 MK299589 MK299772 MK299679 |
Czech Rep. | fulvus | Moravia | Mountain deciduous forest | 48.8550 | 17.6690 | E82 MK266518 MK299591 MK299774 MK299681 |
Czech Rep. | fulvus | Moravia | Mountain spruce forest | 49.5047 | 18.3761 | E83 MK266519 MK299592 MK299775 MK299682 |
Slovakia | fulvus | Kysuce NR | Rich fen with willows | 49.4256 | 18.5255 | E63 MK266506 MK299579 MK299762 MK299669 |
Poland | fulvus | Orava | Fen meadow on a slope | 49.3369 | 19.9055 | E87 MK266522 MK299595 MK299778 MK299685 |
Austria | fulvus | Niederösterreich | Wet brown-moss rich fen | 47.8514 | 15.3895 | E42 MK266496 MK299569 MK299752 MK299659 |
Switzerland | trochiformis | Valais (Alps) | Brown-moss rich fen on a brook margin | 45.9990 | 7.7515 | E1 MK266492 MK299565 MK299748 MK299655 |
Switzerland | trochiformis | Valais (Alps) | Rich fen on a spring | 46.0018 | 7.3407 | E62 MK266505 MK299578 MK299761 MK299668 |
Switzerland | trochiformis | Valais (Alps) | Sloping calcareous fen | 46.0072 | 7.7934 | E50 MK266499 MK299572 MK299755 MK299662 |
Switzerland | fulvus | Bern (Alps) | Sloping calcareous fen with *Schoenus* | 46.5606 | 7.0769 | E64 MK266507 MK299580 MK299763 MK299670 |
Switzerland | trochiformis | Graubünden (Alps) | Brown-moss rich fen | 46.6741 | 10.3523 | E67 MK266509 MK299582 MK299765 MK299672 |
Sweden | fulvus | Jämtland | *Sphagno-Tomentypion* fen | 63.5802 | 15.2311 | E101 MK266534 MK299607 MK299790 MK299697 |
Sweden | praticola | Jämtland | Sloping brown-moss rich fen | 63.5684 | 12.2458 | E66 MK266508 MK299581 MK299764 MK299671 |
Austria | trochiformis | Salzburg (Alps) | Limestone slope | 47.2334 | 13.5067 | E98 MK266532 MK299605 MK299788 MK299695 |
Norway | praticola | Dovrefjell NP | Willow shrubs on a brook margin | 62.3548 | 9.6702 | E90 MK266525 MK299598 MK299781 MK299688 |
Romania | fulvus | Harghita | Wet brown-moss rich fen | 46.3176 | 25.5999 | E44 MK266497 MK299570 MK299753 MK299660 |
Iceland | fulvus | Mývatn | Willow tundra | 65.6289 | -16.9928 | E161 MK266571 MK299644 MK299829 MK299736 |
Iceland | fulvus | Skæftafell NP | Willow tundra | 64.0264 | -16.9779 | E162 MK266572 MK299645 MK299830 MK299737 |
Russia | fulvus | Western Siberia | Mesic birch forest | 56.5067 | 68.4156 | E99 MK266533 MK299606 MK299789 MK299696 |

**Euconulus fulvus egenus**

USA | fulvus | Idaho | Douglas fir forest at base of open talus | 47.6477 | -115.9720 | E112 MK266539 MK299612 MK299795 MK299702 |
USA | fulvus | California | Damp creekside with fern | 34.1792 | -116.9060 | E131 MK266555 MK299628 MK299812 MK299719 |
USA | fulvus | Maine | Rich thuja-ash-red maple swamp | 44.9272 | -67.6589 | E133 MK266556 MK299629 MK299814 MK299721 |
USA | fulvus | Minnesota | Aspen-ash-balsam-spruce forest | 47.6211 | -95.3056 | E134 MK266557 MK299630 MK299815 MK299722 |
USA | fulvus | Texas | Dry rocky oak-juniper forest | 29.2442 | -103.2970 | E135 MK266558 MK299631 MK299816 MK299723 |
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**Euconulus polygyratus**

**Euconulus trochulus** (outgroup)

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Appendix S2. Basic summary statistics of shell measurements and their ratios for the studied *Euconulus* species. Numbers of measured shells/populations: *E. alderi* = 27/7, from Czech Republic, Slovakia, Poland, Bulgaria, Sweden, Russia (Western Siberia) and USA (Alaska); *E. fulvus fulvus* = 16/6, from Czech Republic, Norway, Switzerland and Russia (Western Siberia); *E. fulvus egenus* = 35/12, from Russia (Altai and Western Siberia), Canada (British Columbia and Québec), and USA (Alaska, California, Texas, and Utah), *E. fresti* sp. nov. = 14/4, from USA (Iowa, Maine and Utah) and Canada (British Columbia); *E. polygyratus* = 14/4, from USA (New York, Ohio and Virginia), and Canada (Ontario). Two syntype shells of *E. trochiformis* from Montagu collection (Royal Albert Memorial Museum & Art Gallery, Exeter) and three shells of *E. callopisticus* from Bourguignat collection (Natural History Museum of Geneva) were also included, and eventually classified as *E. f. fulvus*.

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<td>0.36</td>
<td>0.65</td>
<td>0.46</td>
<td>0.57</td>
<td>0.87</td>
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<tr>
<td>Mean</td>
<td>6.05</td>
<td>2656</td>
<td>2198</td>
<td>1414</td>
<td>1227</td>
<td>912</td>
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<td>0.36</td>
<td>0.64</td>
<td>0.46</td>
<td>0.56</td>
<td>0.87</td>
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<td>3rd Quart.</td>
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<td>2320</td>
<td>1486</td>
<td>1288</td>
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<td>0.68</td>
<td>0.47</td>
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<td>0.86</td>
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<tr>
<td>Max</td>
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<td>2900</td>
<td>2539</td>
<td>1557</td>
<td>1336</td>
<td>1012</td>
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<td>0.38</td>
<td>0.70</td>
<td>0.50</td>
<td>0.61</td>
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Appendix S3. Illustration of measurements taken for *Euconulus* shells, as conducted on one of the *E. trochiformis* syntypes.
**Appendix S4.** Mean within-group (A) and between-group (B) distances for *Euconulus fulvus* group taxa in the analysed nDNA and mtDNA constructs, reported as nucleotide base pairs/construct. The lower half of Matrix B represents distances in ITS1+ITS2 and the upper half (demarcated by italic font) represents distances in COII+16S.

### A. Within-group variability

<table>
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<tr>
<th></th>
<th>Mitochondrial</th>
<th>Nuclear</th>
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<tr>
<td><em>E. fresti</em> sp. nov</td>
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<td>0.3</td>
</tr>
<tr>
<td><em>E. alderi</em></td>
<td>3.5</td>
<td>0.4</td>
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<tr>
<td><em>E. fulvus fulvus</em></td>
<td>6.7</td>
<td>1.5</td>
</tr>
<tr>
<td><em>E. fulvus egenus</em></td>
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<td>2.6</td>
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<tr>
<td><em>E. polygyratus</em></td>
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<td>0.3</td>
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### B. Between-group variability

<table>
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<tr>
<th></th>
<th><em>E. fresti</em> sp. nov</th>
<th><em>E. alderi</em></th>
<th><em>E. fulvus fulvus</em></th>
<th><em>E. fulvus egenus</em></th>
<th><em>E. polygyratus</em></th>
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<tr>
<td><em>E. fresti</em> sp. nov</td>
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<td>31.7</td>
<td>27.8</td>
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<tr>
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<tr>
<td><em>E. f. egenus</em></td>
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<td>6.0</td>
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<tr>
<td><em>E. polygyratus</em></td>
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<td>5.2</td>
<td>5.8</td>
<td>2.8</td>
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</table>
Appendix S5. Aligned ITS2 sequence of all analysed Euconulus specimens; the 350T is unique for E. fresti sp. nov. (see the attached fasta file).

For sample codes and specimen characteristics see Appendix S1.

>CTTGAACGCAATATGGCGCCCGTGGGTCCTACATTGGCGACCGGCGACAGGACAGGATGAAATTTGGAATTTTCTCCGACGGTG

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Appendix S6. Terry Frest in the summer of 1989, contemporaneous with his discovery of *E. aff. alderi* in Iowa, USA. (photo courtesy of Edward Johannes).