A NOTE ON THE STATUS OF *GALBA OCCULTA* JACKIEWICZ, 1959 (GASTROPODA: HYGROPHILA: LYMNAEIDAE)

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ABSTRACT: The comparison of the gene nucleotide sequences (mitochondrial cytochrome oxidase c subunit I and *ITS2* fragment of the nuclear rDNA) indicated significant differences within the lymnaeid species of the genus *Ladislavella*. For this reason, we postulate retaining of the name *Ladislavella occulta* (Jackiewicz, 1959) for the molecularly separated lineage of the species occurring in Poland.

KEY WORDS: *COI, ITS2, Ladislavella, L. occulta, L. terebra, Stagnicola, Lymnaea,* taxonomy, classification

INTRODUCTION

JACKIEWICZ (1959) recognised a new species within the complex species *Galba palustris* (O. F. Müller, 1774) and named it *G. occulta*. The species was subsequently assigned to *Stagnicola* Jeffreys, 1830 treated as a subgenus within *Lymnaea* Lamarck, 1799 (JACKIEWICZ 1993, 1998a, 2000) or as a separate genus (FALKNER 1995, FALKNER et al. 2001, GLÖER 2002, GLÖER & MEIER-BROOK 2003). When taxonomic decisions based on the analysis of nucleotide sequences took on significance, MEIER-BROOK & BARGUES (2002), taking into account the length of *ITS2* sequence in *rDNA* gene, included *G. occulta* Jackiewicz in a newly established genus *Catascopia*. They stated after WALTER (1969) that *C. occulta* was an American species introduced in Europe. Later VINARSKI & GLÖER (2008) found that although JACKIEWICZ (1992, 1998b) was aware of the occurrence of *G. occulta* in Siberia, she overlooked that the species discovered by her had been described earlier by WESTERLUND (1885) as *Limnaea palustris* var. *terebra*. VINARSKI (2012) moreover argued that *Ladislavella* B. Dybowski, 1913 was the oldest available name for *Catascopia* Meier-Brook et Bargues, 2002. Finally, *Galba occulta* Jackiewicz, 1959 was assigned as

a junior synonym of *Ladislavella terebra* (Westerlund, 1885) (ANDREYEVA et al. 2010, VINARSKI 2012) and this name was used in subsequent publications (e.g. VINARSKI 2012, PIEŃKOWSKA et al. 2014, 2015a, SCHNIEBS 2016, VINARSKI et al. 2016a, PIECHOCKI & WAWRZYNIAK-WYDROWSKA 2016, SCHNIEBS et al. 2018).

HEBERT et al. (2003a, b) proposed that the nucleotide sequence of the cytochrome oxidase subunit 1 gene (*COI*) could be a marker that would allow to distinguish species, with suggestion that 3% genetic distance could be treated as a threshold between separate taxa at the species level. Usefulness of Hebert's barcoding in taxonomy was supported by many authors (e.g. TAUTZ et al. 2003, GREGORY 2005, PACKER et al. 2009, GOLDSTEIN & DESALLE 2011) with some suggestion that the threshold should be higher for stylommatophoran gastropods (DAVISON et al. 2009, SAUER & HAUSDORF 2012). However SCHNIEBS et al. (2016) decided that *COI* sequences could not be used in stagnicoline lymnaeid taxonomy and excluded this gene from their molecular studies (e.g. SCHNIEBS et al. 2015, 2017, 2018, VINARSKI et al. 2017). On the other hand AKSENOVA et al. (2018) presented a deep

revision of lymnaeid classification with a huge base of *COI* sequences for species identifications.

In this paper we compare *COI* and *ITS2* sequences of *L. terebra* specimens from its Siberian and West Poland populations with the aim of restoring the validity of the taxon described by JACKIEWICZ (1959) to commemorate outstanding achievements in lymnaeid taxonomy of this malacologist who died this year.

MATERIAL AND METHODS

One hundred and sixty six *COI* and 31 *ITS2* sequences were selected from GenBank resources (Appendix 1). They represented the following lymnaeid species: *Ladislavella occulta* (Jackiewicz, 1959), *L. terebra* (Westerlund, 1985), *L. exilis* (Lea, 1834), *L. elodes* (Say, 1821), *L. tumrokensis* (Kruglov et Starobogatov, 1985), *Stagnicola palustris* (O. F. Müller, 1774), *S. corvus* (Gmelin, 1791) and *Lymnaea stagnalis* (Linnaeus, 1758) originating from 61 localities (Appendix 1).

Sequences were prepared using the programme BIOEDIT, version 7.0.5. (HALL 1999). The alignments were performed using the CLUSTAL W programme (THOMPSON et al. 1994) implemented in BIOEDIT. The ends of all sequences were trimmed to obtain four sets of equal length sequences: *COI*, *ITS2* and *COI+ITS2*. The lengths of the sequences after cutting were 558 and 461 bp for *COI*, 488 positions for *ITS2* and 1,014 positions for combined sequences of *COI+ITS2* (558 bp + 456 positions). The sequences were collapsed to haplotypes (*COI*) and to common sequences (*ITS2* and *COI+ITS2*) using the programme ALTER (Alignment Transformation EnviRonment) (GLEZ-PEÑA et al. 2010).

During analysis of the phylogenetic relationships, the sequences were analysed by the genetic distance Neighbour-Joining method (SAITOU & NEI 1987) implemented in MEGA7 (KUMAR et al. 2016) using the Kimura two-parameter model (K2P) for pairwise distance calculations (KIMURA 1980). Best-fit substitution models were calculated using algorithm implemented in MEGA 7 for every set of sequences independently: Tamura 3 parameter evolutionary model (TAMURA 1992) for *COI* alignments, Kimura 2-parameter model (KIMURA 1980) for *ITS2* set and a HKY substitution model for combined data set of *COI+ITS2* (HASEGAWA et al. 1985). For all analyses we assumed a gamma distributed rate variation among sites. Maximum Likelihood analyses were performed using MEGA7.

The ML trees were tested by bootstrap analysis with 1,000 replicates (FELSENSTEIN 1985). In the case of combined alignment *COI+ITS2* parallel Bayesian Interference was conducted using the programme MRBAYES 3.1.2 (RONQUIST & HUELSENBECK 2003). Four Monte Carlo Markov chains were run for 1 million generations, sampling every 100 generations (the first 250,000 trees were discarded as 'burn-in'). This yielded a 50% majority rule consensus tree. Finally, calculated during ML analysis bootstrap values were mapped on the 50% majority rule consensus Bayesian tree.

RESULTS

The *COI* sequences obtained from GenBank were attributed to 77 haplotypes (Appendix 1). They had to be trimmed so that they could be aligned for the construction of ML trees. The tree of 53 haplotypes (558 bp long, COI 1 – COI 53) is shown in Fig. 1. The haplotypes clustered in nine clades, namely *Ladislavella occulta, L. terebra, L. liogyra, L. exilis, L. elodes/tumrokensis, Stagnicola palustris* (1), *S. corvus, S. palustris* (2) and *Lymnaea stagnalis*. The K2P genetic distances within these clades were very small, within a range of 0.0 – 2.0% (mean values) except for *S. palustris* (1) and (2) (when they were treated as one taxon, 4.1%) and *L. stagnalis* (4.4%) (Table 1). The K2P distances between clades were usually larger than 10% when they were compared in pairs (Table 1) with some exceptions, like *L. tumrokensis* vs. *L. elodes* which differed at the level of "within clade" distances (2.3%). Also, smaller distances separated pairs *L. terebra* vs. *L. liogyra* (6.9%), *L. exilis* vs. *L.* *tumrokensis* (6.1%), *L. exilis* vs. *L. elodes* (6.3%) and *S. palustris* (2) vs. *S. corvus* (6.0%). Other K2P distances were larger, however these between clades representing the same genus were smaller (in the range 6.3–15.8% and 7.8% within *Ladislavella* and *Stagnicola,* respectively) than those between clades of different genera (*Ladislavella* vs. *Stagnicola* 18.3–22.0% and *Ladislavella* vs. *Lymnaea* 19.5–20.7%, however the K2P distances were 13.9–15.1% for *Stagnicola* vs. *Lymnaea*).

To compare the above haplotypes with eighty *COI* sequences for *Hinkleya caperata* and one for *L. elodes* deposited in GenBank by MORNINGSTAR et al. (2018) and DEWAARD et al. (2014), respectively (24 additional halotypes COI 54 – COI 77), they all had to be trimmed to 461 bp long haplotypes. The resulting ML tree (Fig. 2) showed similar clades as for longer haplotypes (Fig. 1) with one additional clade of haplotypes characteristic of *H. caperata*.
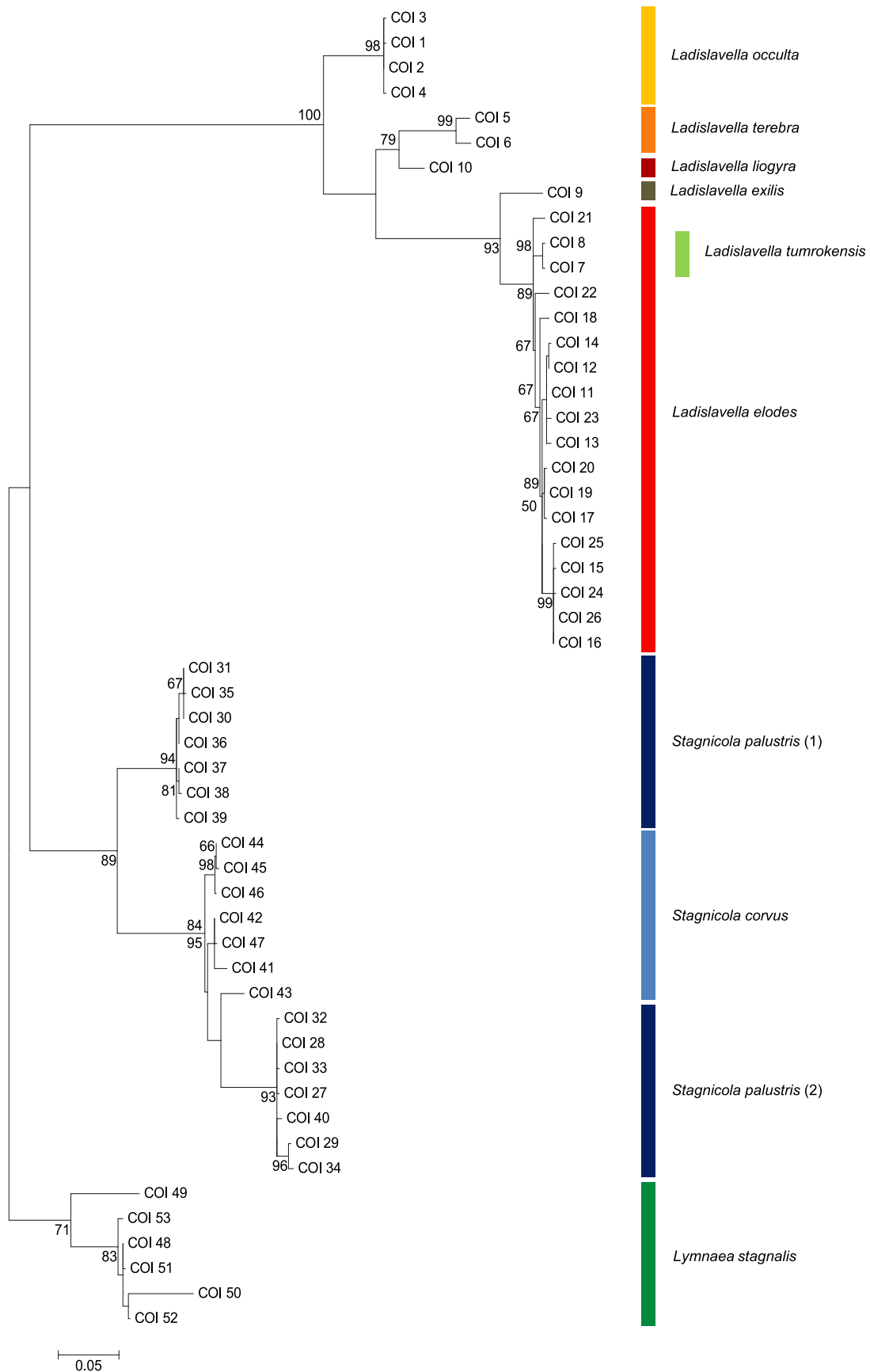
Fig. 1. Maximum Likelihood (ML) tree of the 558-bp-long fragment of *COI* sequences of the studied lymnaeid species with the use of *Lymnaea stagnalis* as outgroup (see Appendix 1). Numbers on branches represent bootstrap support above 50%

The *ITS2* sequences obtained from GenBank were attributed to 23 common sequences (Appendix 1). They clustered in seven clades on the ML tree (Fig. 3), three (*S. corvus*, *S. palustris* and *L. stagnalis*) clearly and four (*L. occulta*, *L. terebra*, *L. elodes* and *L. tum-rokensis*) less separated. The smallest K2P distances (Table 1) differentiated specimens within particular species (mean values up to 1.6%), except *L. terebra* with its intraspecific variation larger (5.3%). The largest K2P distances were between species included

Table 1. Ranges of K2P genetic distances for *COI* and *ITS2* sequences analysed (mean values in parentheses)

| Comparison	COI (%)	ITS2 (%)
Within *L. occulta*	0.2–0.4 (0.3)	0.0
Within *L. terebra*	2.2	5.3
Within *L. liogyra*	0.0	
Within *L. exilis*	0.0	
Within *L. tumrokensis*	0.4	0
Within *L. elodes*	0.2–2.8 (1.4)	0.8
Within *S. palustris*	0.2–11.9 (4.1)	0.2–1.4 (0.9)
Within *S. palustris* (1)	0.2–1.1 (0.6)	
Within *S. palustris* (2)	0.2–1.5 (0.9)	
Within *S. corvus*	0.2–4.1 (2.0)	1.2–2.4 (1.6)
Within *L. stagnalis*	0.7–10.4 (4.4)	0.5–2.3 (1.2)
Between *L. occulta* and *L. terebra*	12.2–12.8 (12.6)	2.4–6.4 (4.4)
Between *L. occulta* and *L. liogyra*	10.0–10.2 (10.2)	
Between *L. occulta* and *L. exilis*	15.0–15.5 (15.3)	
Between *L. occulta* and *L. tumrokensis*	15.0–15.5 (15.3)	6.8
Between *L. occulta* and *L. elodes*	14.5–16.9 (15.8)	6.8–7.1 (7.0)
Between *L. occulta* and *S. palustris*	19.7–21.4 (20.5)	29.8–32.1 (31.0)
Between *L. occulta* and *S. corvus*	18.5–20.9 (19.6)	31.0–31.8 (31.5)
Between *L. occulta* and *L. stagnalis*	18.5–21.4 (19.9)	34.0–34.4 (34.1)
Between *L. terebra* and *L. liogyra*	6.9	
Between *L. terebra* and *L. exilis*	12.3–13.4 (12.9)	
Between *L. terebra* and *L. tumrokensis*	13.7–15.1 (14.4)	6.2–10.3 (8.3)
Between *L. terebra* and *L. elodes*	13.5–16.1 (15.1)	6.2–10.3 (8.1)
Between *L. terebra* and *S. palustris*	20.6–22.8 (21.6)	32.0–38.6 (35.4)
Between *L. terebra* and *S. corvus*	19.9–21.3 (20.5)	32.2–39.0 (35.5)
Between *L. terebra* and *L. stagnalis*	18.7–22.6 (20.6)	35.5–40.7 (37.9)
Between *L. liogyra* and *L. exilis*	11.9	
Between *L. liogyra* and *L. tumrokensis*	12.1	
Between *L. liogyra* and *L. elodes*	12.3–13.0 (12.7)	
Between *L. liogyra* and *S. palustris*	20.6–21.8 (21.1)	
Between *L. liogyra* and *S. corvus*	20.4–22.1 (20.8)	
Between *L. liogyra* and *L. stagnalis*	18.5–21.6 (20.0)	
Between *L. exilis* and *L. tumrokensis*	6.1	
Between *L. exilis* and *L. elodes*	5.5–6.7 (6.3)	
Between *L. exilis* and *S. palustris*	18.9–19.7 (19.4)	
Between *L. exilis* and *S. corvus*	18.0–19.0 (18.3)	
Between *L. exilis* and *L. stagnalis*	17.5–21.6 (19.5)	
Between *L. tumrokensis* and *L. elodes*	1.8–2.8 (2.3)	0.5–0.8 (0.6)
Between *L. tumrokensis* and *S. palustris*	21.2–22.4 (21.8)	32.5–34.9 (34.0)
Between *L. tumrokensis* and *S. corvus*	20.7–21.4 (21.1)	34.9–36.6 (35.8)
Between *L. tumrokensis* and *L. stagnalis*	19.9–22.4 (20.8)	36.7–38.1 (37.1)
Between *L. elodes* and *S. palustris*	20.2–23.5 (22.0)	32.2–34.6 (33.7)
Between *L. elodes* and *S. corvus*	19.9–22.7 (21.2)	34.6–36.3 (35.5)
Between *L. elodes* and *L. stagnalis*	19.0–23.1 (20.7)	35.6–38.1 (36.5)
Between *S. palustris* and *S. corvus*	5.1–11.6 (7.8)	5.0–6.5 (5.9)
Between *S. palustris* and *L. stagnalis*	11.8–17.4 (13.9)	9.5–12.3 (10.8)
Between *S. corvus* and *L. stagnalis*	14.0–17.1 (15.1)	12.2–13.8 (13.1)
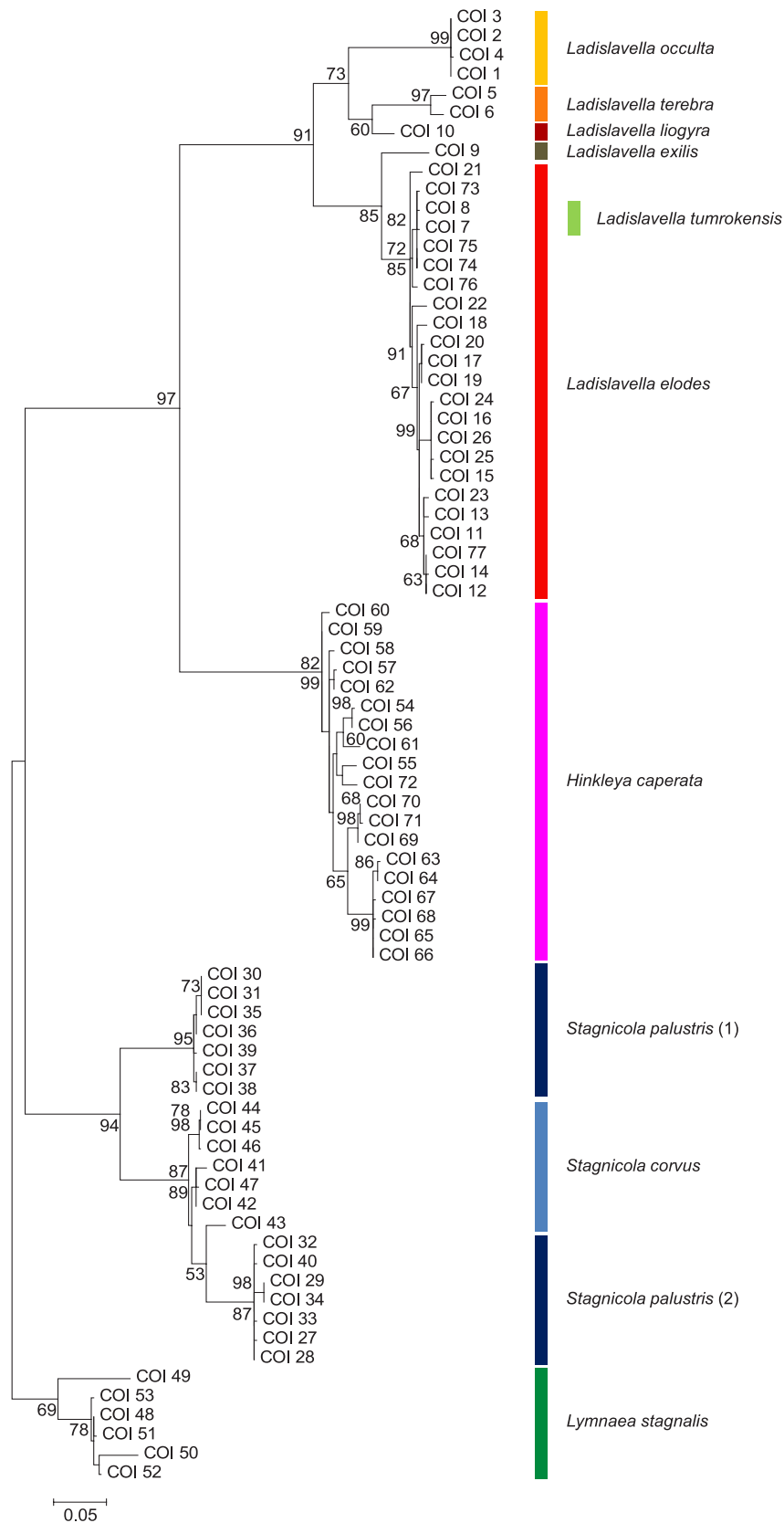
Fig. 2. Maximum Likelihood (ML) tree of the 461-bp-long fragment of *COI* sequences of the studied lymnaeid species with the use of *L. stagnalis* as outgroup (see Appendix 1). Shortening the length of the sequences within alingment allowed to add sequences of *Hinkleya caperata* to the phylogenetic analysis. Numbers on branches indicate bootstrap support above 50%
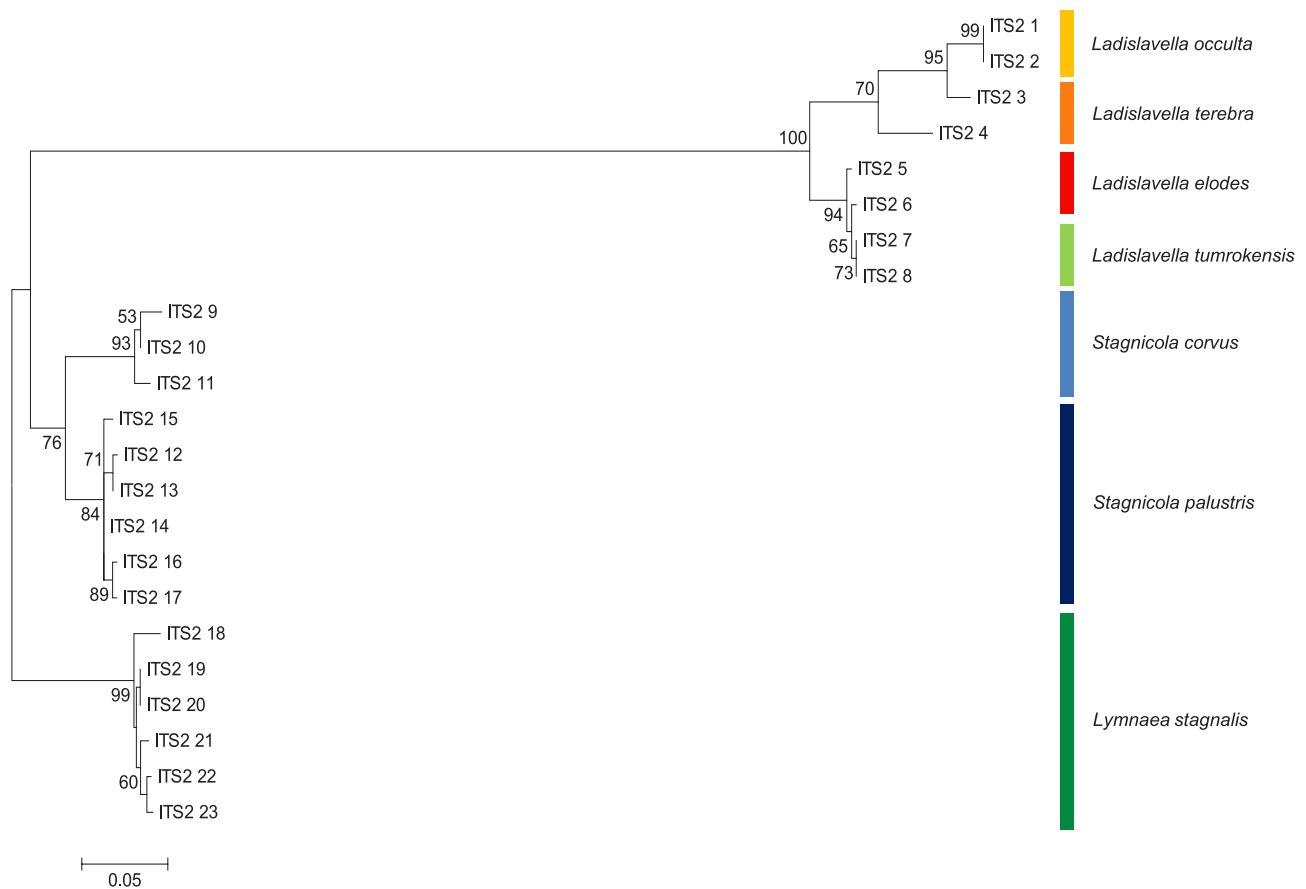
Fig. 3. Maximum Likelihood (ML) tree of the 488-position-long fragment of *ITS2* sequences of the studied lymnaeid species with the use of *L. stagnalis* as outgroup (see Appendix 1). Numbers on branches indicate bootstrap support above 50%

Table 2. Number of differences in *ITS2* nucleotide sequences between analysed species of genus *Ladislavella*

	Number of nucleotide sequence differences			
	Total	Substitutions		Insertions + deletions
		Transitions	Transversions	
L. occulta (ITS2 1 & 2) vs. *L. terebra* (ITS2 3 & 4)	43–59	2–11	7–13	19–50
L. occulta (ITS2 1 & 2) vs. *L. elodes* (ITS2 5 & 6)	80–86	11–12	12–13	56–61
L. occulta (ITS2 1 & 2) vs. *L. tumrokensis* (ITS2 7 & 8)	87–89	11	13–14	62–64
L. terebra (ITS2 3 & 4) vs. *L. elodes* (ITS2 5 & 6)	62–89	13–20	9–17	28–66
L. terebra (ITS2 3 & 4) vs. *L. tumrokensis* (ITS2 7 & 8)	68–91	12–19	10–19	30–69
L. elodes (ITS2 5 & 6) vs. *L. tumrokensis* (ITS2 7 & 8)	4–10	0–2	1–2	2–7

Table 3. Combined sequences of *COI* and *ITS2* fragments for Bayesian analysis

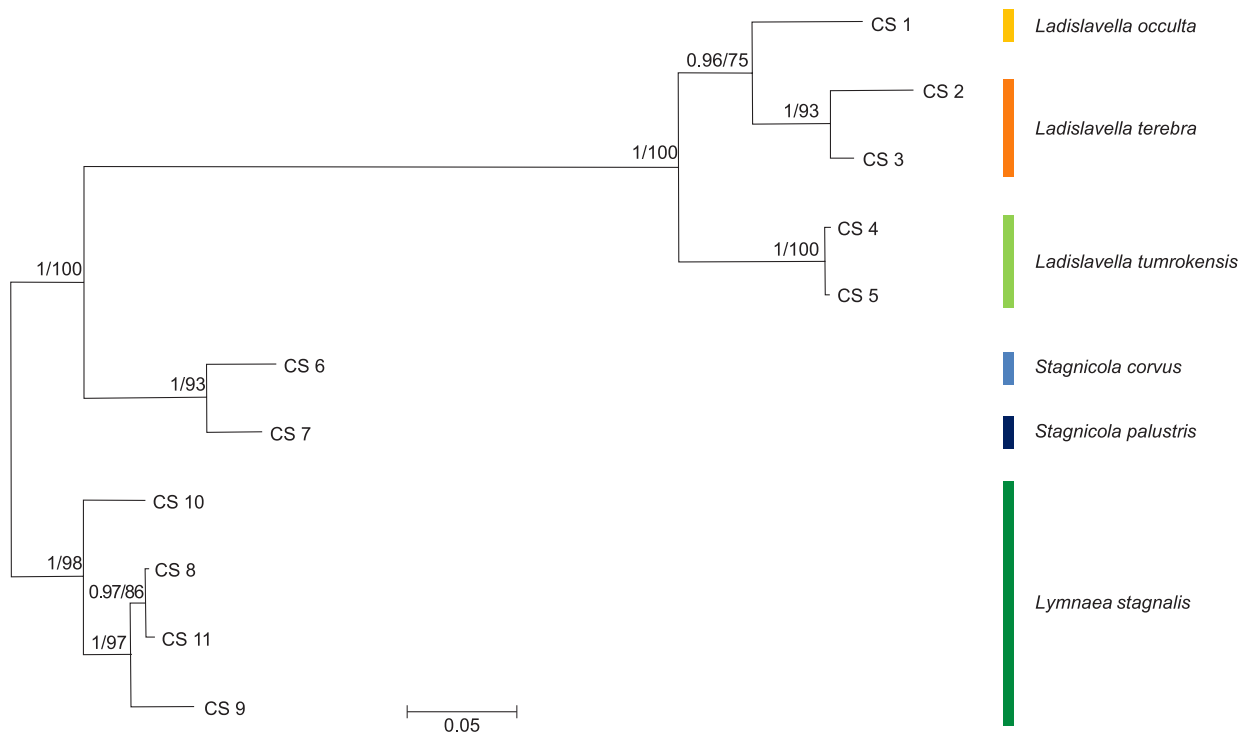| Species	Combined sequences	*COI*	*ITS2*
Ladislavella occulta	CS 1	KP070796	KP070772
Ladislavella terebra	CS 2	LT623591	HE613324
	CS 3	LT623592	HE613325
Ladislavella tumrokensis	CS 4	KP830102	KP830108
	CS 5	KP830103	KP830109
Stagnicola corvus	CS 6	KP070781	KP070771
Stagnicola palustris	CS 7	KP070773	KP070769
Lymnaea stagnalis	CS 8	FR797867	FR797836
	CS 9	HG932251	HG931958
	CS 10	HG932247	HG931952
	CS 11	HG932255	HG931965

Fig. 4. Majority-rule consensus tree obtained from Bayesian Inference analysis (BI) of the combined data set of *COI* and *ITS2* DNA sequences (see Table 3) of the studied lymnaeid species. The tree was rooted with *L. stagnalis* combined sequences. Posterior probabilities (left) and bootstrap support above 50% from ML analysis (right) are marked on branches

in *Stagnicola* and *Lymnaea* clades and those grouped in four *Ladislavella* clades analysed in pairs (mean values 31.0–37.9%). Mean values of the K2P distance within *Ladislavella* species support their separation (*L. occulta* vs. *L. terebra* 4.4%, *L. occulta* vs. *L. tumrokensis* 6.8%, *L. occulta* vs. *L. elodes* 7.0%, *L. terebra* vs. *L. tumrokensis* 8.3%, *L. terebra* vs. *L. elodes* 8.1%), except a pair *L. tumrokensis* and *L. elodes* (mean K2P distance 0.6%). It is noteworthy that the number of differences between *ITS2* sequences of *L. occulta* and *L. terebra* is smaller than those distinguishing *L. occulta* from *L. elodes* and *L. tumrokensis* as well as *L. terebra*

from *L. elodes* and *L. tumrokensis* (Table 2). However it is much larger than the number differences in *ITS2* sequences between *L. elodes* and *L. tumrokensis* (Table 2) and much smaller than between *L. occulta* or *L. terebra* and species of *Stagnicola* or *Lymnaea* (data not shown).

Ten *ITS2* sequences deposited in GenBank for lymnaeids made it possible to create combined sequences *COI + ITS2* (Table 3). The Bayesian inference tree resulted in six well separated clades *L. occulta, L. terebra, L. tumrokensis, S. palustris, S. corvus* and *L. stagnalis* (Fig. 4).

DISCUSSION

No differences were found in the structure of the shell and reproductive system between topotypical material of *Galba occulta* Jackiewicz and Siberian *Limnaea palustris* var. *terebra* Westerlund in careful comparative studies (VINARSKI 2003, 2012, VINARSKI & GLÖER 2008). Moreover, specimens of both species were closely related based on their *ITS2* sequences in *rDNA* gene (VINARSKI et al. 2016a). Therefore VINARSKI & GLÖER (2008) synonymised the taxa giving a priority to the older name. Somewhat later VINARSKI (2012) assigned it to the genus *Ladislavella* B. Dybowski, 1913 as *L. terebra* (Westerlund, 1885).

We found that the specimens from the Polish population in Gorzykowo near Gniezno (W. Poland)

differed in their nucleotide sequences of *COI* and *ITS2* fragments from the two Siberian populations (Tjumen Region and Altai Republic, Russia) (Figs 1–4). The K2P distances between the common sequences of *ITS2* fragment suggest that these populations are closely related (as suggested by VINARSKI et al. 2016a). However the differences of *ITS2* sequences between the Altai and Tjumen populations require further in-depth research on a larger number of populations. On the other hand, K2P distances between the haplotypes COI 1 – COI 4 and COI 5 & COI 6, representing these two groups (Polish and Siberian), respectively, are much higher (12.2–12.8%) than the 3% threshold established by HEBERT et al. (2003a,

b) for species delimitation. We are aware of the objections to the use of Hebert's threshold (Davison et al. 2009, Sauer & Hausdorf 2012 and references cited therein). Moreover we have always stressed (Pieńkowska et al. 2015b, 2018a, b) that molecular features alone are not enough for species designation and that they have to be supported by anatomical features. However, the Polish and Siberian populations are far away from each other and one can expect a process of speciation that has a faster expression in the features of the rapidly evolving mitochondrial genome (Thomaz et al. 1996, Remigio & Hebert 2003) than, for example, in the features of shell and genitalia. Therefore we propose to retain the species name introduced by Jackiewicz (1959), i.e. *L. occulta*, for the Polish lineage of the taxon, especially when the other valid species of the genus *Ladislavella* differ to a lesser extent in the nucleotide sequence of the *COI* gene than *L. occulta* and *L. terebra* (see Table 1).

L. occulta or *L. terebra* were identified on the basis of shell and genital system features from several localities from Europe (West Poland, South Sweden, Czech Republic, Bosnia-Hercegovina, Ukraine) and Siberia (Yeniseysk and Selenga River near Baikal lake) by Jackiewicz (1992, 1993, 1997, 1998a, b) (who used the name *Lymnaea* (*Stagnicola*) *occulta*); several localities in Germany and Rusia (especially in Siberia and Far East) were added to its distribution by Vinarski & Glöer (2008) and recently from Ukraine (Khust district) by Anistratenko et al. (2018) (using the name *Ladislavella terebra*). However *L. occulta* or *L. terebra* at these localities were identified on the basis of shell and genital system features. We report differences in *COI* and *ITS2* sequences between one Polish (Gorzykowo, W. Poland) and two Russian (Siberia) localities. Further molecular research on *L. occulta* and *L. terebra* populations from other localities is necessary. Unfortunately all but one (Gorzykowo) Polish localities found by Jackiewicz and her co-workers (Jackiewicz 1959, 1993, 1998a, 2000) were destroyed, so the Polish *L. occulta* lineage is threatened with extinction (Rybska et al. 2007).

Although this was not the aim of this study, we add a few remarks about lymnaeid taxa resulting from our analysis of the *COI* gene sequences:

1. *COI* gene sequences well support the generic classification of the following taxa: *Ladislavella* B. Dybowski, 1913, *Hinkleyia* F. C. Baker, 1928, *Stagnicola* Jeffreys, 1830 and *Lymnaea* Lamarck, 1799.
2. The results of this paper support the suggestion that *L. tumrokensis* and *L. elodes* are conspecific (according to Vinarski et al. 2017 they represent two subspecies of *Ladislavella catascopium*).
3. The status of *Stagnicola palustris* should be verified. We found that this species was represented by two different *COI* lineages. Schniebs et al. (2016) pointed to the inconsistency of stagnicoline lymnaeid classification based on mitochondrial sequences vs. that resulting from the analysis of nuclear genes and reproductive system anatomy. Drawing conclusions on the basis of our results would be premature. Further research is needed on a larger number of *S. palustris* populations to determine if this difference in *COI* sequences is a result of interspecific hybridisation or speciation visible in the mitochondrial genome.
4. Although *Lymnaea stagnalis* is a well defined species, it is also greatly diversified in *COI* nucleotide sequences which suggests that further studies on its populations would be necessary.

ACKNOWLEDGEMENTS

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APPENDIX 1

GenBank sequences of *COI* and *ITS2* gene fragments selected for analysis with data on localities where the specimens were collected

No.	Country, region, site	Geographic coordinates	Species	COI haplotype	COI ## GenBank	ITS2 common sequence	ITS2 ## GenBank	References for GenBank deposits
1.	Poland: Gorzykowo, near Września-Gniezno	52°24'50.0"N 17°43'13.6"E	*Ladislavella occulta*	COI 1 COI 2 COI 3 COI 4	KP070796 KP070797 KP070798 KP070799	ITS2 1	KP070772	Pieńkowska et al. 2015a
2.	Russia: Siberia, Tjumen Region, vicinity of Labytnangi	66°38'54.1"N 66°25'25.3"E	*Ladislavella terebra*	COI 5	LT623591	ITS2 2 ITS2 4	AJ457042 HE613322 HE613323 HE613324	Bargues et al. 2003 Schniebs et al. 2018 Vinarski et al. 2016a
3.	Russia: Siberia, Altai Republic, Lake Teletzkoje	51°47'29.5"N 87°16'56.1"E	*Ladislavella terebra*	COI 6	LT623592	ITS2 3	HE613325 HE613326	Schniebs et al. 2018
4.	Russia: Kamchatka, Tumrok, Verkhne-Schapinskiye	55°12'02"N 160°23'10"E	*Ladislavella tumrokensis*	COI 7 COI 8	KP830103 KP830104 KP830107 KP830102 KP830106 KP830105	ITS2 7 ITS2 8	KP830109 KP830110 KP830108 KP830112 KP830111	Vinarski et al. 2016b
5.	USA: Illinois, Jersey County, Ditch along the Stump Lake access road	39°01'25.7"N 90°33'29.8"W	*Ladislavella exilis*	COI 9	HM230364			Campbell et al. 2017
6.	Russia: Primorsky Krai	unpublished	*Ladislavella liogyra*	COI 10	MH190007			Aksenova et al. 2018
7.	Canada: Alberta, Banff NP; Vermillion Lakes /Bow River	51°10'40.8"N 115°36'21.6"W	*Ladislavella elodes*	COI 11	KM612124 KM612184			DeWaard et al. 2014
8.	Canada: Alberta, Banff NP; Cave and Basin Area	51°10'15.6"N 115°35'09.6"W	*Ladislavella elodes*	COI 13	KM612224 KM611916			DeWaard et al. 2014
9.	Canada: British Columbia, Yoho NP, Emerald Lake Trials	51°26'34.8"N 116°32'31.2"W	*Ladislavella elodes*	COI 12 COI 14	KM611837 KM612105 KM612117 KM612123 KM612154 KM612215 KM611972			DeWaard et al. 2014
10.	Canada: Manitoba	58°45'18.0"N 93°54'54.0"W	*Ladislavella elodes*	COI 15	HQ926943 HQ926945			iBOL Data Release 2011

No.	Locality — Country, region, site	Geographic coordinates	Species	COI haplotype	COI ## GenBank	ITS2 common sequence	ITS2 ## GenBank	References for GenBank deposists
11.	Canada: Manitoba	58°45'32.4"N 93°57'07.2"W	*Ladislavella elodes*	COI 16	HQ926954 HQ926955			IBOL DATA RELEASE 2011
12.	Canada: Yukon Territory, Kluane National Park, Dezadeash River Trail	60°45'03.6"N 137°31'48.0"W	*Ladislavella elodes*	COI 16	MF544168			DeWAARD 2017
13.	Canada: Manitoba	58°39'47.2"N 94°09'58.3"W	*Ladislavella elodes*	COI 16 COI 17	HQ926942 HQ926930 HQ926934 HQ926939 HQ926969			IBOL DATA RELEASE 2011
				COI 18	HQ926929 HQ926931			
				COI 21 COI 22	HQ926965 HQ926933			
14.	Canada: Alberta, Jasper NP, Range Road 275a, wetland	53°12'00.0"N 117°54'46.8"W	*Ladislavella elodes*	COI 19	MF544604 MF544617			DeWAARD 2017
				COI 20	MF544232 MF544367			
15.	Canada: Alberta, Lacombe County	52°16'12.0"N 113°34'48.0"W	*Ladislavella elodes*	COI 23	KT831386			GORDY et al. 2016
16.	Canada: Manitoba	58°43'47.6"N 93°46'49.8"W	*Ladislavella elodes*	COI 26	HQ926912 HQ969867			IBOL DATA RELEASE 2011
17.	Canada: Ontario, Bruce Peninsula National Park, Scugog Lake	45°07'11.6"N 81°31'55.6"W	*Ladislavella elodes*	COI 25 COI 26	MF545071 MF544921			DeWAARD 2017
18.	USA: California, Lassen County, Ash Creek at Ash Valley Rd, SE. of Adin	41°05'24.8"N 120°42'50.8"W	*Ladislavella elodes*			ITS2 5	LN851520	VINARSKI et al. 2017
19.	USA: Oregon, Malheur County, Crooked Spring at Crooked Spring Rest Area	42°47'57.9"N 117°44'46.6"W	*Ladislavella elodes*			ITS2 6	LN851519	VINARSKI et al. 2017
20.	Germany: Saxony, wetland west of Burghausen	51°21'33"N 12°1'444"E	*Stagnicola palustris*	COI 27	FR797869 FR797870			VINARSKI et al. 2011
21.	Germany: Baden-Württemberg, lake Bodensee, peninsula Mettnau, north side	47°43'52"N 09°00'004"E	*Stagnicola palustris*	COI 28	FR797871 FR797872			VINARSKI et al. 2011
22.	Poland: lake Wilczyńskie, near Konin.	52°29'03"N 18°07'24"E	*Stagnicola palustris*	COI 28	KP070774	ITS2 15	HE577631	SCHNIEBS et al. 2012 PIEŃKOWSKA et al. 2015a
23.	Germany: Mecklenburg-Western Pomerania, lake Grosser Plaetschsee, south bank	53°2625"N 12°19'18"E	*Stagnicola palustris*	COI 28	LN515542			SCHNIEBS et al. 2016
24.	Poland: ponds at Morasko near Poznań	52°28'14"N 16°55'42"E	*Stagnicola palustris*	COI 29 COI 32	KP070777 KP070773			PIEŃKOWSKA et al. 2015a

No.	Country, region, site	Geographic coordinates	Species	COI haplo-type	COI ## GenBank	ITS2 common sequence	ITS2 ## GenBank	References for GenBank deposists
25.	Russia: Tjumen Region, Yamal Peninsula, ox-bow of river Shchuchya*	67°23'N 67°36'E*	*Stagnicola palustris*	COI 29	HG932232 LN515547			VINARSKI et al. 2014
26.	Russia: Nenets Autonomous Okrug	unpublished	*Stagnicola palustris*	COI 29	MH189888			AKSENOVA et al. 2018
27.	France	unpublished	*Stagnicola palustris*	COI 30	HQ660032			DAYRAT et al. 2011
28.	Germany: Saxony, Dresden, small pond	51°00'22.5"N 13°42'24.5"E	*Stagnicola palustris*	COI 31	LN515546			SCHNIEBS et al. 2016
29.	Poland: lake Mrowinko, Santoczno near Gorzów Wlkp.	52°51'15"N 15°19'38"E	*Stagnicola palustris*	COI 33	KP070775		KP070769**	PIEŃKOWSKA et al. 2015a
30.	Poland: lake Wysokie Brodno	53°18'08.6'N 19'2159.0'E	*Stagnicola palustris*	COI 34	KP070776			PIEŃKOWSKA et al. 2015a
31.	Germany: Saxony, Dresden, pond Zschoner Mühlteich	51°03'33"N 13°38'23"E	*Stagnicola palustris*	COI 35	LN515541			SCHNIEBS et al. 2016
32.	Germany: Mecklenburg-Western Pomerania, lake Koelpinsee	53°30'50.0"N 12°36'42.0"E	*Stagnicola palustris*	COI 36	LN515543			SCHNIEBS et al. 2016
33.	Germany: Saxony, old ox-bow of river Röder between Rödern and Oberrödern	51°14'01"N 13°42'02"E	*Stagnicola palustris*	COI 37 COI 38	LN515544 LN515545			SCHNIEBS et al. 2016
34.	Russia: Moscow City, a pond in Nekrasovka District	55°41'32.52"N 37°570.68"E	*Stagnicola palustris*	COI 39	LN515548 LN515549			SCHNIEBS et al. 2016
35.	Germany: Brandenburg, Lake Schulzensee near Chorin	52°54'19.38"N 13°52'14.71"E	*Stagnicola palustris*	COI 40	LT623589 LT623590	ITS2 12	LT623578	SCHNIEBS et al. 2016
36.	France: Normandie, Tatihou Island, Contentin Germany: Bavaria, Danube valley The Netherlands: Province Friesland, Beetsterzwaag	unpublished	*Stagnicola palustris*			ITS2 13	AJ319620	BARGUES et al. 2001
37.	Sweden: Norrköping	58°37'12.0"N 16°22'48.0"E	*Stagnicola palustris*			ITS2 14	KC248373	NOVOBILSKY et al. 2013
38.	Russia: Tjumen Region, Yamal Peninsula, ox-bow of river Shchuchya*	67°23'N 67°36'E*	*Stagnicola palustris*			ITS2 16	HG931946	VINARSKI et al. 2014
39.	Germany: Mecklenburg-Vorpommern, lake Grosser, Plaetschsee, south bank	53°26'25"N 12°19'18"E	*Stagnicola palustris*			ITS2 17	FR797838	VINARSKI et al. 2011
40.	Germany: Baden-Württemberg, lake Illmensee	47°51'43.07"N 9°22'40.60"E	*Stagnicola corvus*			ITS2 9	LN515551	SCHNIEBS et al. 2016
41.	Germany: Saxony, Niederspree, pond Großer Tiefzug*	51°24'20"N 14°53'38"E*	*Stagnicola corvus*	COI 41	HG932236			VINARSKI et al. 2014
42.	Germany: Saxony, Grethen, ditch on the westside of the pond Kleiner Kirchenteich*	51°14'29"N 12°39'22"E*	*Stagnicola corvus*	COI 42	HG932237 HG932238	ITS2 10	HE577638	VINARSKI et al. 2014 SCHNIEBS et al. 2012 VINARSKI et al. 2014
43.	Germany, Mecklenburg-Western Pomerania, small pond near Neperstorf*	unpublished	*Stagnicola corvus*	COI 43	HG932239			VINARSKI et al. 2014

No.	Locality — Country, region, site	Geographic coordinates	Species	COI haplotype	COI ## GenBank	ITS2 common sequence	ITS2 ## GenBank	References for GenBank deposists
44.	Poland: Luboń, water bodies near railroad close to Kocie doły	52°19'53"N 16°53'22"E	*Stagnicola corvus*	COI 44	KP070781			PIEŃKOWSKA et al. 2015a
45.	Poland: stream Niwnica, around Nysa	50°26'50"N 17°23'43"E	*Stagnicola corvus*	COI 45 COI 46	KP070782 KP070783		KP070771**	PIEŃKOWSKA et al. 2015a
46.	Austria: Wallersee, near Salzburg	unpublished	*Stagnicola corvus*			ITS2 11	AJ319625	BARGUES et al. 2001
47.	Germany: Saxony, pond Vierteich near Freitelsdorf	51°15'43"N 13°41'57"E	*Lymnaea stagnalis*	COI 47	LN515538			SCHNIEBS et al. 2016
48.	Germany: Baden-Württemberg, Konstanz-Egg, ditch Hockgraben	47°40'57.3"N 9°11'34.2"E	*Lymnaea stagnalis*	COI 48	FR797867	ITS2 19	FR797834	VINARSKI et al. 2011
49.	Germany: Baden-Württemberg, lake Bodensee, peninsula Mettnau, north side,	47°43'52"N 09°00'04"E	*Lymnaea stagnalis*	COI 49	HG932247	ITS2 20	FR797836	VINARSKI et al. 2011
50.	Germany: Mecklenburg-Western Pomerania: Krümmel, Lake Tralowsee*	unpublished	*Lymnaea stagnalis*	COI 50	HG932251	ITS2 21	HG931952	VINARSKI et al. 2014
51.	France: vicinity of Toulouse*	unpublished	*Lymnaea stagnalis*	COI 51	HG932255	ITS2 23	HG931958	VINARSKI et al. 2014
52.	Bulgaria: Plovdiv, floodplain of the Mariza River*	unpublished	*Lymnaea stagnalis*			ITS2 18	HG931965	VINARSKI et al. 2014
53.	Germany: Saxony, Dresden-Zschieren, old branch of river Elbe	50°59'50"N 13°52'28"E	*Lymnaea stagnalis*			ITS2 22	HE573064	SCHNIEBS et al. 2011
54.	Poland: Bystrzyca River, Spiczyn	51°20'29"N 22°44'51"E	*Lymnaea stagnalis*	COI 52	KP070784			PIEŃKOWSKA et al. 2015a
55.	Poland: pond, Niemcza	50°43'30"N 16°49'32"E	*Lymnaea stagnalis*	COI 53	KP070795			PIEŃKOWSKA et al. 2015a
56.	USA: Indiana, Posey County, floodplain of the Wabash River near New Harmony (NH)	unpublished	*Hinkleya caperata*	COI 54 COI 55 COI 56 COI 57 COI 58 COI 59 COI 60 COI 61 COI 62	MF962171 MF962172 MF962173 MF962181 MF962185 MF962186 MF962174 MF962175 MF962176 MF962177 MF962178 MF962180 MF962183 MF962179 MF962182			MORNINGSTAR et al. 2018

No.	Locality		Species	COI		ITS2		References for GenBank deposists
	Country, region, site	Geographic coordinates		haplo-type	## GenBank	common sequence	## GenBank	
57.	USA: New Mexico, Chaves County, Hunter Marsh on Bitter Lake National Wildlife Refuge (BLHM)	unpublished	*Hinkleya caperata*	COI 63	MF962187 MF962193 MF962197 MF962201			MORNINGSTAR et al. 2018
				COI 64	MF962188 MF962202			
				COI 65	MF962189 MG962192 MF962200			
				COI 66	MF962190 MF962191 MF962196 MF962198			
				COI 67	MF962194 MF962199			
				COI 68	MF962195			
58.	USA: Texas, Pecos County, Diamond Y Preserve (DY)	unpublished	*Hinkleya caperata*	COI 69	MF962203 MF962208 MF962209 MF962211 MF962215 MF962216			MORNINGSTAR et al. 2018
				COI 70	MF962204 MF962206 MF962207 MF962210 MF962212 MF962213 MF962214 MF962217			
				COI 71	MF962205			

| No. | Locality | | Species | COI | | ITS2 | | References for GenBank |
	Country, region, site	Geographic coordinates		haplo-type	## GenBank	common sequence	## GenBank	deposits
59.	USA: New Mexico, Sandoval County, vernal grassland pools in Valles Caldera National Preserve (VC)	unpublished	*Hinkleya caperata*	COI 66	MF962223 MF962226			MORNINGSTAR et al. 2018
				COI 72	MF962218 MF962219 MF962220 MF962221 MF962222 MF962224 MF962225 MF962227 MF962228 MF962229 MF962230 MF962231 MF962232 MF962233 MF962234 MF962235 MF962236			
60.	USA: New Mexico, Taos County, snow-melt pools near Big Costilla Peak (BCP)	unpublished	*Ladislavella elodes*	COI 73	MF962156 MF962158 MF962159 MF962160 MF962161 MF962162			MORNINGSTAR et al. 2018
				COI 74	MF962157 MF962166			
				COI 75	MF962167 MF962169			
				COI 76	MF962163 MF962164 MF962165 MF962168 MF962170			
61.	Canada: British Columbia, Yoho NP, Emerald Lake Trials	51°2634.8″N 116°32′31.2″W	*Ladislavella elodes*	COI 77	KM612042			DEWAARD et al. 2014

* Data published due to courtesy of KATRIN SCHNIEBS (Senckenberg Natural History Collections Dresden, Germany).
** ITS2 sequences too short for ML tree presented on Fig. 3.