Chinese mystery snail, *Cipangopaludina chinensis,* phylogenetic analysis and barcoding

Pevensey Levels, Sussex; and Southampton, Hampshire

Natural England Commissioned Report NECR555

August 2024

Chinese mystery snail © Gavin Measures

www.gov.uk/natural-england

About Natural England

Natural England is here to secure a healthy natural environment for people to enjoy, where wildlife is protected and England's traditional landscapes are safeguarded for future generations.

Further Information

This report can be downloaded from the Natural England Access to Evidence Catalogue. For information on Natural England publications or if you require an alternative format, please contact the Natural England Enquiry Service on 0300 060 3900 or email enquiries@naturalengland.org.uk.

Copyright

Catalogue code: NECR555

Report details

Author(s)

Helen C. Rees and Gavin H. Measures

Natural England Project Manager

Gavin Measures Gavin.Measures@naturalengland.org.uk

Contractor

RSK ADAS Ltd.

Keywords

Cipangopaludina chinensis, *Cipangopaludina cathayensis*, Chinese mystery snail, eDNA, invasive, invertebrate, snail, phylogenetic, freshwater, ditches

Acknowledgements

We would like to thank Gavin Measures of Natural England for commissioning the project and provision for snail specimens for analysis.

Citation

This report should be cited as: REES, H.C., MEASURES, G.H., 2024. Chinese mystery snail, *Cipangopaludina chinensis,* phylogenetic analysis and barcoding. Pevensey Levels, Sussex and Southampton, Hampshire NECR555. Natural England.

Foreword

Chinese mystery snail (*Cipangopaludina chinensis*) is a problem invasive non-native species in many parts of the world and was first found in the UK within ditches at Pevensey Levels, Sussex in 2018 and at two lakes at Southampton Common, Hampshire in 2022.

This project was commissioned by Natural England to provide additional information on the identification of invasive snail species found at Pevensey Levels and Southampton Common SSSIs. A total of 15 snail specimens were collected from these sites and an additional site near the New Forest and identified by DNA sequence analysis and phylogenetic work to ensure that the species was correctly identified at all sites where it is present. This information will be used to provide evidence for a request by Natural England to have *Cipangopaludina chinensis* added to the list of invasive species of special concern under the Invasive Alien Species Regulation which will allow a ban on keeping or selling of this species in the Great Britain.

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Executive summary

The Chinese mystery snail (*Cipangopaludina chinensis*) is an invasive species that poses a risk to our native aquatic invertebrate communities in the UK. Early detection of invasive species is key to controlling populations and environmental DNA methods provide the opportunity detect rare species without using traditional monitoring methods.

This study was commissioned to build on the qPCR assay developed for detecting *C. chinensis* by ADAS in 2021 (NECR410) (naturalengland.org.uk), Rees et al. 2023). The qPCR successfully distinguished between *C. chinensis* and other co-occurring species. The only exception to this was *Cipangopaludina cathayensis* a very closely related species. *C. chinensis* and *C. cathayensis* are distinguishable via various anatomical characteristics (Lu et al. 2014) but have very similar COI sequences which prevented the development of species-specific primers.

To confirm the species identity at both Pevensey Levels and Southampton sites as the taxonomically identified *C. chinensis*, Natural England collected a total of 15 specimens for DNA sequencing (Cytochrome Oxidase subunit I gene (COI)) and phylogenetic analysis.

Sequences generated were submitted to BLAST using default parameters and BOLD using 'species level barcode records' for species identification.

Phylogenetic trees were created in BOLD (www.barcodinglife.org) and in MEGA version 11 (www.megasoftware.net). MEGA was used to allow all specimens to be included in a single phylogenetic tree constructed with various Bellamya snail species (five); Cipangopaludina snail species (four), Viviparus snail species (two), Margarya snail species (two), *Pleuropoma jana* was used as an outgroup (Wang et al, 2017).

This study has shown the specimens collected from Pevensey and Southampton to be a very close match to sequences belonging to *Cipangopaludina chinensis*. It should be noted that there is still some uncertainty involved in the identification of the Pevensey and Southampton specimens so we would also recommend that longer sequences and/or complete mitochondrial genomes are sequenced to try and improve the specimen identification as the sequences used within this study were relatively short.

Contents

Introduction

Natural England is the Government's advisor for the natural environment. It provides practical advice on how to safeguard England's natural wealth for the benefit of everyone. RSK ADAS is an environmental consultancy which exists to provide ideas, specialist knowledge and solutions to secure our food and enhance the environment.

In 2021, Natural England commissioned a project with RSK ADAS to develop an eDNA assay for the detection of *Cipangopaludina chinensis* (Gray, 1834), also known as the Chinese mystery snail. This invasive species is a problem in many parts of the world (Global Invasive Species Database, 2021) and was first found in the UK within ditches at the Pevensey Levels, Sussex in September 2018 (Willing, 2021a).

C. chinensis[1] the Chinese mystery snail or 'trapdoor snail' is a large freshwater snail native to East Asia. The 'trapdoor' refers to an oval plate (operculum) which seals the aperture of the snail when the snail is fully retracted. Two subspecies of C. chinensis that are recognised are: *C. chinensis chinensis* (Gray, 1834) and *C. chinensis malleata* (Reeve, 1863; also known as *C. chinensis laeta*) (Matthews et al. 2017) the latter of which was found to be present at the Pevensey Levels, Sussex during the 2021 eDNA study.

Survey work carried out in July 2019 found the presence of a recruiting population of the snail in an approximately 400m stretch of the ditch. Further surveys carried out at this ditch system in February 2021 found several smaller juveniles which suggested that a breeding population was present (Willing 2021a). Confirmation of the breeding population was made during eDNA surveys in August 2021 when specimens were collected and returned to the laboratory. During tissue removal for DNA extraction one of the specimens was found to contain juvenile snails (Figure 1) - this species is known to give live birth during June to October potentially having more than 160 young in their lifetime (Jokinen, 1992).

[1] *Cipangopaludina chinensis* is adopted following MolluscaBase (MolluscaBase 2021)

Figure 1. Images of *C. chinensis.* Left hand side: specimen collected from Pevensey Levels August 2021 prior to DNA extraction (© Helen Rees, ADAS). Middle: specimens removed from Pevensey Levels August 2021 (© Gavin Measures, NE). Right hand side: specimen cut open during DNA extraction to reveal juveniles (© Helen Rees, ADAS).

During 2022, reports were received that the shells of *C. chinensis* had been found at Southampton Common SSSI, likely when the boating lake was drained. The site contains two large artificial lakes that support nationally important breeding assemblage of amphibians. A manual survey of the two lakes on Southampton Common SSSI and at a third lake (Cemetery Lake) took place on the 24th and 25th August 2022 entailing visual inspection and netting. Evidence of Chinese mystery snail was found during this survey with multiple adult and juvenile shells and adult specimens being found in both the Boating Lake (sites 1-4) and Ornamental Lake (sites 5-8). eDNA analysis utilising the qPCR assay for *C. chinensis* previously developed by RSK ADAS (Rees et al. 2022; NECR410) on water samples collected at this time found that 6 out of the 7 sites sampled were positive. Since 2022, two further sites in the Southampton area have been confirmed for the presence of Chinese mystery snail: one site at Marchwood (New Forest); the other near the River Test.

The qPCR developed to detect *C. chinensis* (Rees et al. 2022; NECR410, Rees et al. 2023) successfully distinguished between *C. chinensis* and other co-occurring species in that no cross-amplification was detected either *in silico* or *in vitro*. The only exception to this was *Cipangopaludina cathayensis* a very closely related species found to cross-amplify during *in silico* testing which would be likely to cross-amplify if tested with this assay. *C. chinensis* and *C. cathayensis* are distinguishable via various anatomical characteristics (Lu et al. 2014) but have very similar COI sequences which prevented the development of species-specific primers. We were unable to source DNA from *C. cathayensis* species to test the qPCR assay *in vitro*, however, as the species was not believed to be present in the UK this would not affect the application of the qPCR assay. To confirm the species identity of specimens collected at both Pevensey and Southampton as the taxonomically identified *C. chinensis*, Natural England collected specimens at both locations for sequencing analysis. The results of this study will be used in an application by Natural England to have *C. chinensis* listed as a species of special concern.

Aims and Objectives

The overall aim of this study was to DNA sequence and confirm the species identity of 15 snail specimens collected from the Pevensey Levels and Southampton. This report details the methodology employed in this study, the results obtained and, discussion of these results.

Materials and Methods

Specimen Collection

Putative *C. chinensis* snail specimens were collected from ditches at Pevensey Levels by Natural England in August 2021 during a period of dry weather with no recent rainfall. Specimens were collected from locations in Southampton Common in August 2022 and February 2023 and in Marchwood in October 2023. Specimens were preserved in 95% ethanol prior to couriering to the ADAS laboratories. The 95% ethanol preservative was replaced after 1 day and 1 week (Ben Price, personal communication).

At each survey location in the infested ditch or lake, the search for snails entailed:

1. Initial visual searches of the margins for evidence of the snails which appear to favour shallow vegetated edges.
2. For deeper water sediments (the snails are on the bottom) a framed survey net fitted with a 2mm mesh. This allowed rapid exit of sediments, but retention of even the smallest newly born *C. chinensis.*
3. Sediment samples were washed through a large (30 cm diameter) course 2-tier (10mm / 2mm) sieve-nest, again to allow large samples to be processed quickly and with the retention of even juvenile *C. chinensis.*
4. All captured *C. chinensis* were retained and stored in 95% ethanol for later laboratory analysis.

Table 1. Specimens collected

Site	Specimen ID
Pevensey Levels, Sussex	1-6, F, M
Southampton Common, Hampshire	OL4 – Ornamental Lake BL – Boating Lake
Marchwood (New Forest), Hampshire	M1-M5

Laboratory Standard and Specifications

All laboratory activities associated with DNA analysis are subject to errors if quality control is inadequate. Our DNA analysis follows a unidirectional workflow with separate laboratories and staff to act as a physical separation for the different aspects of the

analysis work. This greatly reduces the potential for contamination of samples or the PCR amplicons. ‘Blank’ PCRs (sterile water rather than DNA) are used to monitor for reagent/procedural contamination, and in addition positive control samples are used to increase confidence in the results and identify any cross-contamination issues, should they occur.

DNA extraction

Each snail specimen was individually transferred to a clean, sterile petri dish and a photographic record made. A fresh sterile scalpel blade was used to remove a small piece of tissue (20 mg) from each specimen and mash the tissue into a paste before placing into a sterile 1.5 mL Eppendorf tube. DNA was extracted from snail specimens using DNeasy blood and tissue kit (Qiagen) following the manufacturer’s instructions. Briefly, 180µL ATL and 20µL PK buffer was added to each specimen and incubated for 1-2 hours at 56°C until completely digested. Final DNA elution/resuspension was in 200µL AE buffer for all specimens.

All DNA extracts were quantified using a Qubit 3.0 Fluorometer (Invitrogen) following the manufacturer’s instructions then stored at -20 ºC prior to PCR set up.

Specimen identification PCR

All PCR set up was performed in a clean ‘PCR room’ within a UV sterilisable cabinet within a separate laboratory to DNA extraction using dedicated equipment and PPE. To ensure the unidirectional workflow DNA extracts are collected from the DNA extraction laboratory and transferred to the PCR set-up laboratory.

PCRs were performed to confirm the identity of the provided specimens using the LCO1490/HCO2198 primer combination (Folmer et al. 1994).

LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'
HC02198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

These primers amplify an approximately 710 base pair fragment of the Cytochrome Oxidase subunit I gene (COI) commonly used for barcoding.

PCRs were set up in a total volume of 25 µL consisting of:

a. 3 µL of extracted template DNA at 3 ng/µl,
b. 2.5 µL of each primer (0.4 µmol/L),
c. 12.5 µL of Itaq (BioRad) Sybr Green mastermix
d. 4.5 µL ddH2O.

Each sample was run in duplicate on a Bio-Rad CFX Connect real-time PCR machine as follows: an initial incubation for 1 minute at 95ºC; followed by 35 cycles with a melting temperature of 95°C for 1 minute; an annealing temperature of 40ºC and a final extension step at 72ºC for 90 seconds before holding at 4ºC until collection of PCR products for analysis.

PCR amplicons were purified using the Nucleospin® Gel and PCR Cleanup kit (Machery-Nagel) following the manufacturer's instructions and sent for Sanger sequencing in both directions using a 1 in 10 dilution of each of the PCR primers (Source Bioscience).

Specimen identification and phylogenetics

Returned Sanger sequence data was cross checked in BioEdit version 7.2.5 before forward and reverse sequences were aligned using EMBL's Omega (multiple sequence alignment), now replaced with EMBL's Job Dispatcher. A consensus sequence was generated from the sequence alignment and then submitted to BLAST using default parameters and BOLD using 'species level barcode records' for species identification.

Phylogenetic trees were created in BOLD and in MEGA version 11. BOLD automatically generated a phylogenetic tree upon use of the identification engine using a Kimura 2 parameter distance model (Kimura 1980). A phylogenetic tree was therefore generated for each individual specimen. MEGA was used to allow all specimens to be included in a single phylogenetic tree constructed with various *Bellamya* snail species (five); *Cipangopaludina* snail species (four), *Viviparus* snail species (two), *Margarya* snail species (two) from China, *Pleuropoma jana* was used as an outgroup (Wang et al, 2017). All sequence accession numbers are shown in Appendix 3. All sequences were recovered from BOLD and Genbank. *C. cathayensis* sequence NC_025577.1 was 100% identical to the complete mitochondrial sequence KM503121 (Yang et al. 2016) and was chosen for use in this study as it was added to BOLD / GenBank more recently. A maximum likelihood statistical method was used along with a Kimura 2 parameter distance model. The maximum likelihood method uses statistical methods to assign probabilities to all possible phylogenetic trees for a given group of input sequences.

Results and Discussion

There has been considerable debate on the taxonomy of the *Cipangopaludina* genus which is part of the Viviparidae family of large operculate freshwater snails sometimes known as mystery snails. To ascertain the species identity of 15 specimens collected at Pevensey and Southampton, a sequencing and phylogenetic approach was carried out.

Aligned consensus sequences for each of the 15 specimens had an average length of 626 base pairs after poor quality sequence was removed from either end of the generated sequence and are shown in **Appendix 1**, along with a percentage similarity matrix produced by Clustal. Sequence identification results produced by BLAST and BOLD searches are shown in Table 2. All results produced by BLAST were for *Cipangopaludina cathayensis* with the two different matched sequences coming from complete mitochondrial genomes. When specimen sequences were run through BOLD, all sequences (except 2 Pevensey which was identified as *C. cathayensis*) were identified as *C. chinensis*. Through investigation of the BOLD record numbers for the top hits it was found that none of the sequences were present within the Genbank database that BLAST

uses to assign sequence identity. The sequence for 2 Pevensey was of poor quality and considerable editing had to be performed using the sequencing chromatograms illustrated through BioEdit. A repeat of the PCR and Sanger sequencing did not lead to any improvement thus was excluded from further analysis. DNA from 2a Pevensey, the offspring of 2 Pevensey (see Figure 1 right hand side) was Sanger sequenced instead as it would be the same species as the parent.

Table 2. Results of BLAST and BOLD searches

Specimen	BLAST top hit (accession number)	Percentage identity	BOLD top hit (BOLD record number)	Percentage identity*
1 Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	98.06% over 100% of sequence	*Cipangopaludina chinensis* (BFMN105-18)	99.22%
2a Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	98.76% over 100% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	98.74%
4 Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	98.15% over 100% of sequence	*Cipangopaludina chinensis* (BFMN105-18)	99.22%
5 Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	97.26% over 99% of sequence	*Cipangopaludina chinensis* (BFMN099-18)	99.82%
6 Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	98.06% over 99% of sequence	*Cipangopaludina chinensis* (BFMN105-18)	99.43%
F Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	97.87% over 100% of sequence	*Cipangopaludina chinensis* (BFMN105-18)	98.45%
M Pevensey	*Cipangopaludina cathayensis* (KX688549.1)	98.14% over 100% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	99.32%
BL Southampton	*Cipangopaludina cathayensis* (KX688549.1)	97.55% over 97% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	99.54%
OL4 Southampton	*Cipangopaludina cathayensis* (NC_025577.1)	97.79% over 100% of sequence	*Cipangopaludina chinensis* (BFMN105-18)	98.67%
M1 Southampton	*Cipangopaludina cathayensis* (KX688549.1)	97.14% over 99% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	99.54%
M2 Southampton	*Cipangopaludina cathayensis* (KX688549.1)	98.36% over 100% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	99.14%

Specimen	BLAST top hit (accession number)	Percentage identity	BOLD top hit (BOLD record number)	Percentage identity*
M3 Southampton	Cipangopaludina cathayensis (KX688549.1)	97.38% over 98% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	99.54%
M4 Southampton	*Cipangopaludina cathayensis* (KX688549.1)	97.43% over 98% of sequence	*Cipangopaludina chinensis* (BFMN110-18)	99.38%
M5 Southampton	*Cipangopaludina cathayensis* (KX688549.1)	97.00% over 98% of sequence	*Cipangopaludina chinensis* (BFMN099-18)	99.68%

*BOLD does not specify the percentage of the sequence that was used to make the identification hence no values are stated for 'over % of sequence'.

Examples of individual specimen phylogenetic trees from the various sampling locations (1 Pevensey, 2a Pevensey, BL, OL4, and M1) generated via BOLD are shown in **Appendix 2**. Specimen sequences are outlined with a solid black line, Genbank sequences are outlined with a dashed black line, and all other sequences are from BOLD. BOLD notes that the latter Genbank sequences may be less up to date in terms of sequence accuracy and taxonomic identification. The majority of the sequences from individual specimens clustered with *C. chinensis* sequences found in BOLD. The sequence from 2a Pevensey specimen clustered with both the *C. chinensis* sequences from BOLD but also *C. cathayensis*. This was unexpected for sample 2a whose top hit on BOLD was *C. chinensis*.

A phylogenetic tree produced in MEGA for all specimens with various *Bellamya* and *Cipangopaludina* species from China is shown in Figure 2. The tree with the highest log likelihood (log likelihood is a negative number) is shown as a value closer to zero for example -977.53, is better than -3433.71. Because you want to show the phylogenetic tree that is most probable you need to maximize the log-likelihood. Again, specimen sequences clustered with *C. chinensis* sequences from BOLD, however, *C. cathayensis* was also found in the same cluster, matching particularly well to OL4 and not 2a Pevensey as might have been expected from the BOLD phylogenetic trees. The *C. chinensis* sequence with accession number NC_035734.1 did not cluster with the group of *C. chinensis* sequences found on the BOLD database nor the specimen sequences and it is unclear as to why this is the case. These results illustrate that there is still some uncertainty as to the identity of the specimens although it is likely that they are all *C. chinensis*.

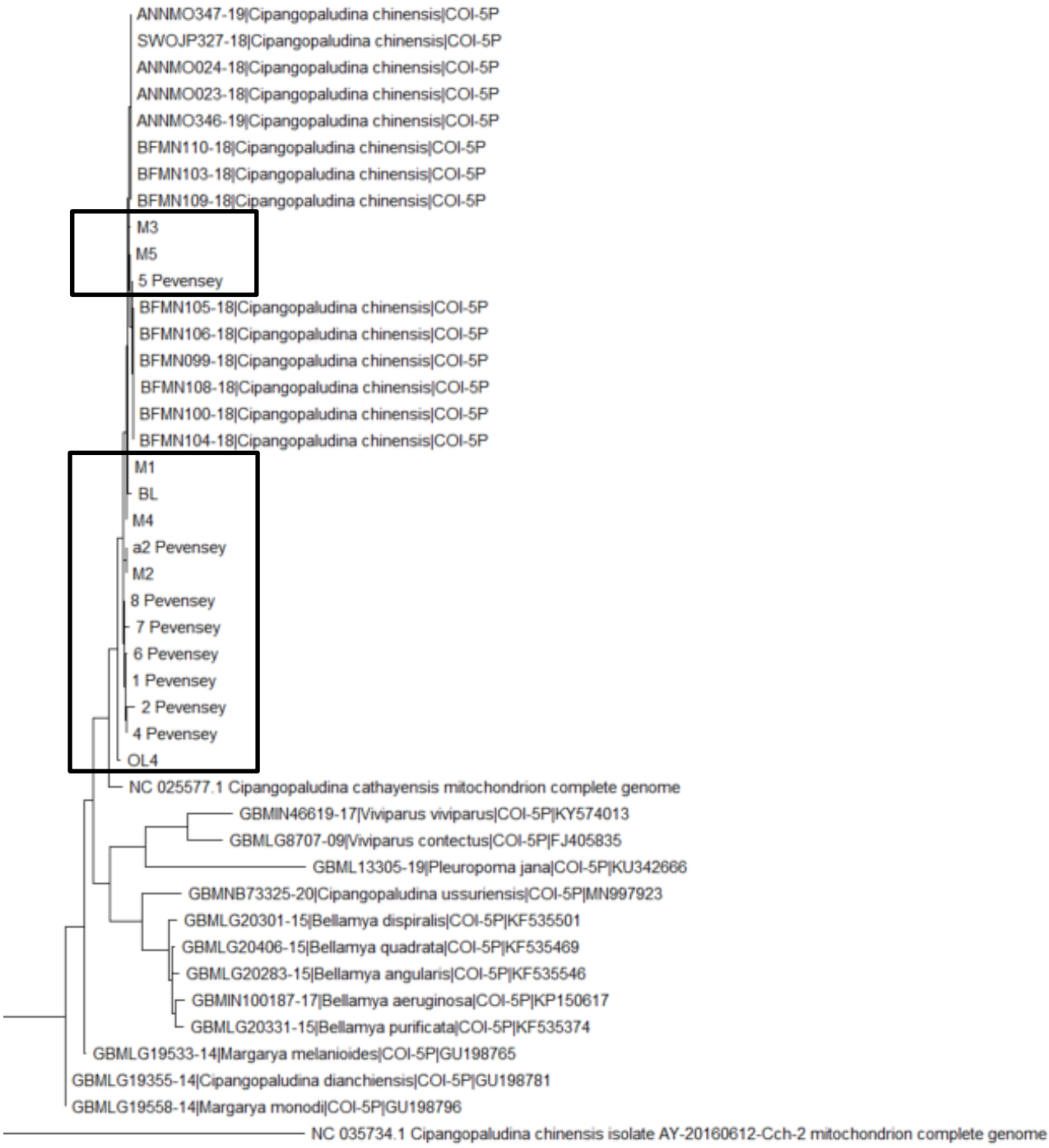


Figure 2. Phylogenetic tree of collected specimens alongside *Bellamya, Margarya, Viviparus* and *Cipangopaludina* snail species (MEGA output). Specimen sequences are outlined with a solid black line, Genbank sequences are outlined with a dashed black line, and all other sequences are from BOLD.

Recommendations

This study has shown the specimens collected from Pevensey and Southampton to be a very close match to sequences belonging to *Cipangopaludina chinensis,* therefore we would recommend that this species is added to the list of species of special concern by

Natural England. It should be noted that there is still some uncertainty involved in the identification of the Pevensey and Southampton specimens so we would also recommend that longer sequences and/or complete mitochondrial genomes are sequenced to try and improve the specimen identification as the sequences used within this study were relatively short. It may also be possible to determine whether the introductions in Southampton were from a single introduction or multiple introductions by utilising microsatellite analysis.

List of Figures

List of Tables

References

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R., VRIJENHOEK, R., (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3(5): 294-9.

GLOBAL INVASIVE SPECIES DATABASE, (2021). Species profile: *Cipangopaludina chinensis*. Downloaded from http://www.iucngisd.org/gisd/species.php?sc=1812 on 06-12-2021.

GRAY, J. E., (1834). *Paludina chinensis*. Pg 599 and Plate IV in Cuvier's Animal Kingdom, Volume 12, Mollusca and Radiata (E. Griffith & E. Pidgeon, eds.) Whittaker & Co, London.

JOKINEN, E. H., (1992). The freshwater snails (Mollusca: Gastropoda) of New York State. The University of the State of New York, The State Education Department, The New York State Museum, Albany, New York.

KIMURA, M., (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111-120.

MATTHEWS, J., COLLAS, F.P.L., DE HOOP, L. VAN DER VELDE, G., LEUVEN, R.S.E.W., (2017). Risk assessment of the alien Chinese mystery snail (*Bellamya*

chinensis). Series of Reports Environmental Science, Institute for Water and Wetland Research, Radboud University.

REES, H.C., MEASURES, G.H., KANE, S.D., MADDISON, B.M., (2022). Developing eDNA techniques for the detection of *Cipangopaludina chinensis* Chinese mystery snail in ditch systems at Pevensey Levels, Sussex, 2021. Natural England Commissioned Reports, NECR410.

REES, H.C., KANE, S.D., MEASURES, G.H., (2023). Quantitative PCR (qPCR) assay for the specific detection of the Chinese mystery snail (*Cipangopaludina chinensis*) in the UK. PLoS ONE 18(10): e0292163. https://doi.org/10.1371/journal.pone.0292163

REEVE, L. A., (1863). Monograph of the genus Paludina. Conchologica Iconica Volume 14 (1864), unpaginated. Lovell Reeve & Co, London.

WANG, J-G., ZHANG, D., JAKOVLIC, I., WANG, W-M., (2017). Sequencing of the complete mitochondrial genomes of eight freshwater snail species exposes pervasive paraphyly within the Viviparidae family (Caenogastropoda). PLoS ONE 12(7): e0181699. https://doi.org/10.1371/journal.pone.0181699

WILLING, M., (2021a). A survey to assess the status of the Chinese Mystery Snail *Cipangopaludina chinensis* & the Little Whirlpool Ram's-horn snail *Anisus vorticulus* in a ditch & it's connecting channels on Glynleigh Level, Pevensey Levels (February – March 2021). Report to Natural England.

WILLING, M., (2021b). Appendix Notes to accompany: A survey to assess the status of the Chinese Mystery Snail *Cipangopaludina chinensis* & the Little Whirlpool Ram's-horn snail *Anisus vorticulus* in a ditch & it's connecting channels on Glynleigh Level, Pevensey Levels (February – March 2021). August 2021. Report to Natural England.

WILLING, M., (2022). A survey to assess the presence of the Chinese Mystery Snail *Cipangopaludina chinensis* in relation to the recent placement of three coffer dams & resurvey of a site supporting the Little Whirlpool Ram's-horn snail *Anisus vorticulus* on Glynleigh Level, Pevensey Levels, April 2022. Report to Natural England.

YANG, H., ZHANG, J.E., LUO, H., LUO, M., GUO, J., DENG, Z., ZHAO, B., (2016). The complete mitochondrial genome of the mudsnail *Cipangopaludina cathayensis* (Gastropoda: Viviparidae). Mitochondrial DNA Part A 27, https://doi.org/10.3109/19401736.2014.971274

Appendix 1a: aligned consensus sequences

>1 Pevensey

TCTACAAATCATAAAGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTGGTTGG
TACTGGATTAAGAATTTTAATTCGGATTGAACTTGGTCAACCTGGTACTTTATTGGGAGATGAT
CAGTTGTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGCCTATA
ATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTTTTC
CTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAGCTG
CTGTGGAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAGCAC
ATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATTTTA
GGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCGGT
TGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAGTGTTA
GCAGGGGCAATTACTATATTATTAACTGATCGAAA

>2 Pevensey

GTATATTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTGGATTGAGAATTTTAATTCGGATT
GAACTTGGTCAACCTGGTACTTTATTGGGAGATGATCAGTTGTACAATGTNATTGTAACCGCT
CATGCCTTTGTTATAATTTTTTTTTTAGTTATGCCTATAATAATTGGNGGGTTTGGAAATTGATT
GATTCCTTTAATATTAGGAGCTCCAGATATAGCTTTTCCTCGTTTAAATAATATAAGTTTTTGAT
TATTACCTCCTAGGTTATTACTTCTTTTATCTTCAGCTGCTGTGGAAGGAGGTGTTGGGACTGG
TTGAACAGTATATCCTCCTTTAGCTGGAAATTTAGCACATGCTGGTGGTTCAGTTGATTTAGCT
ATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATTTTAGGGGCTGTTAATTTTATTACTACTGT
AATTAATATACGTTGATGTGGTATACAATTTGAGCGGTTGCCGTTGTTTGTATGATCTGTGAAA
ATTACTGCTATTTTGCTTTTACTGTCTTTACCAGTGTTAGCAGGGGCAATTACTATATTATTAAC
TGATCGAAATTTTAATACGTCTTTTTTCGATCTGC

>2A Pevensey

AATTTTTTTTTTAGTTATGCCTATAATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATT
GGGAGCTCCAGATATAGCTTTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGT
TATTGCTTCTTTTATCTTCAGCTGCTGTGGAAGGGGGTGTTGGGACTGGTTGAACAGTATATC
CTCCTTTAGCTGGAAATTTAGCACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCA
TTTAGCTGGTGCATCTTCTATTTTAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTT
GATGTGGTATACAATTTGAGCGGTTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTT
GCTTTTACTGTCTTTACCAGTGTTAGCAGGGGCAATTACTATATTATTAACTGATCGAAATTTTA
ATACGTCTTTTTTCGATCCTGCTGGTGGTGG

>4 Pevensey

GAACTTGGTATATTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTGGATTAAGAATTTTAATT
CGGATTGAACTTGGTCAACCTGGTACTTTATTGGGAGATGATCAGTTGTACAATGTAATTGTAA
CTGCTCATGCATTTGTTATAATTTTTTTTTTTAGTTATGCCTATAATAATTGGTGGGTTTGGAAAT
TGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTTTTCCTCGTTTAAATAATATAAGTTT
TTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAGCTGCTGTGGAAGGAGGTGTTGGG
ACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAGCACATGCTGGTGGTTCAGTTGATT
TAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATTTTAGGGGCTGTTAATTTTATTACT
ACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCGGTTGCCGTTGTTTGTATGATCTG
TGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAGTGTTAGCAGGGGCAATTACTATATTA
TTAACTGATCGAA

>5 Pevensey

AATAATAAAGATATTGGAACTTGGTATATTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTG
GATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAGATGATCAGTT
GTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTTAGTTATGCCTATAATAAT
TGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTTTTCCTCGT
TTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAGCTGCTGTG
GAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAGCACATGCT
GGTGGTTCAGTTGATTTAGCTATTTTTTCACTGCATTTAGCTGGTGCATCTTCTATTTTAGGGG
CTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCGGTTGCCG
TTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCGGTGTTAGCAGG
GGCAATTACTATATTATTAACTGATCGAAATTTTAATACAT

>6 Pevensey

AATCATAAAGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTG
GATTAAGAATTTTAATTCGGATTGAACTTGGTCAACCTGGTACTTTATTGGGAGATGATCAGTT
GTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTTAGTTATGCCTATAATAAT
TGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTTTTCCTCGT
TTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAGCTGCTGTG
GAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAGCACATGCT
GGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATTTTAGGGG
CTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCGGTTGCCG
TTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCGGTGTTAGCAGG
GGCAATTACTATATTATTAACTGATCGAAATTTTAATACAT

>F Pevensey

TCAGGATTGGTTGGTACTGGATTGAGAATTTTAATTCGGATTGAGCTTTGTCAACCTGGTACTT
TATTGGGAGATGATCAGTTGTACAATGTAATTGTAACTGCTCACGCATTTGTTATAATTTTTTTT
TTAGTTATGCCTATAATAAGTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTC
CAGATATAGCTTTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTT
CTTTTATCTTCAGCTGCTGTGGAAGGGGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTA
GCTGGAAATTTAGCACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTG
GTGCATCTTCTATTTTAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGT
ATACAATTTGAGCGGTTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACT
GTCTTTACCAGTGTTAGCAGGGGCAATTACTATATTATTAACTGATCGA

>M Pevensey

TTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTGGATTAAGAATTTTAATTCGGATTGAGCT
TGGTCAACCTGGTACTTTATTGGGAGATGATCAGTTGTACAATGTAATTGTAACTGCTCATGCA
TTTGTTATAATTTTTTTTTTTAGTTATGCCTATAATAATTGGTGGGTTTGGAAATTGATTGATTCCT
TTAATATTGGGAGCTCCAGATATAGCTTTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACC
TCCTAGGTTATTGCTTCTTTTATCTTCAGCTGCTGTGGAAGGAGGTGTTGGGACTGGTTGAAC
AGTGTATCCTCCTTTAGCTGGAAATTTAGCACATGCTGGTGGTTCAGTTGATTTAGCTATTTTT
TCATTGCATTTAGCTGGTGCATCTTCTATTTTAGGGGCTGTTAATTTTATTACTACTGTAATTAA
TATACGTTGATGTGGTATACAATTTGAGCGGTTGCCGTTGTTTGTATGATCTGTGAAAATTACT
GCTATTTTGCTTTTACTGTCTTTACCAGTGTTAGCAGGGGCAATTACTATATTATTAACTGATCG
AAATTTTAAAC

> BL Southampton

GGTCAACCAAATCATAAAGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTGG
TTGGTACTGGATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAGA
TGATCAGTTGTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGCC
TATAATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCT
TTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCA
GCTGCTGTGGAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTA
GCACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTA
TTTTAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAG
CGGTTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAGT
GTTAGCAGGGGCAATTACTATATTATTAACTGATCGAAATTTTAATACATCTTTTTTCGATCCTG
CCGGTGGTGGTGATCCTATTTTATACCAACATTTATTTTGATTTTTTGGTCACCCTGAAAGTTT

>OL4 Southampton

CATAAAGATATTGGAACTTGGTATATTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTGGGT
TAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAGATGATCAGTGGTA
CAAAGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGCCTATAATAATTGG
TGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCTGATATAGCTTTTCCTCGTTTA
AATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTACTTCTTTTATCTTCAGCTGCTGTGGA
AGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAGCACATGCTGG
TGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATTTTAGGGGCTG
TTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCGGTTGCCGTTG
TTNGTATGATCTGTGAAAATTACTGCT

>M1 Southampton

GGTCCACAAATCATAAAGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTGGT
TGGTACTGGATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAGAT
GATCAGTTGTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGCCT
ATAATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTT
TTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAG
CTGCTGTGGAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAG
CACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATT
TTAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCG
GTTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAGTGT
TAGCAGGGGCAATTACTATATTATTAACTGATCGAAATTTTAATACATCTTTTTTCGATCCTGCC
GGTGGTGGTGATCCTATTTTATACCAACATTTATTTTGATTTTTTGGTCACC

>M2 Southampton

AATCATAAGGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTGGTTGGTACTG
GATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAGATGATCAGTT
GTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGCCTATAATAAT
TGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTTTTCCTCGT
TTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAGCTGCTGTG
GAAGGGGGTGTTGGGACTGGTTGAACAGTATATCCTCCTTTAGCTGGAAATTTAGCACATGCT
GGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCTATTTTAGGGG
CTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCGGTTGCCG
TTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAGTGTTAGCAGG
GGCAATTACTATATTATTAACTGATCG

>M3 Southampton

TTTGGTCAANAAATCATAAAGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTG
GTTGGTACTGGATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAG
ATGATCAGTTGTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGC
CTATAATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGC
TTTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTC
AGCTGCTGTGGAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTT
AGCACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCACTGCATTTAGCTGGTGCATCTTCT
ATTTTAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGA
GCGATTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAG
TGTTAGCAGGGGCAATTACTATATTATTAACTGATCGAAATTTTAATACATCTTTTTTCGATCCT
GCCGGTGGTGGTGATCCTATTTTATACCAACATTTATTTTGATTTTTTGGTCC

>M4 Southampton

TTTGGTCAACAAATCATAAAGATATTGGAACTTTGTATATTTTGTTTGGTATGTGATCAGGATTG
GTTGGTACTGGATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAG
ATGATCAGTTGTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGC
CTATAATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGC
TTTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTC
AGCTGCTGTGGAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTT
AGCACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCATTGCATTTAGCTGGTGCATCTTCT
ATTTTAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGA
GCGGTTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCA
GTGTTAGCAGGGGCAATTACTATATTATTAACTGATCGAAATTTTAATACGTCTTTTTTCGATCC
TGCCGGTGGTGGTGATCCTATTTTATACCAACATTTATTTTGATTTTTTGGTCACCCTGAAAGT
TTAAA

>M5 Southampton

TCAACAAATCATAAAGATATTGGAACTTGGTATATTTTGTTTGGTATGTGATCAGGATTGGTTG
GTACTGGATTAAGAATTTTAATTCGGATTGAGCTTGGTCAACCTGGTACTTTATTAGGAGATGA
TCAGTTGTACAATGTAATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTTTAGTTATGCCTAT
AATAATTGGTGGGTTTGGAAATTGATTGATTCCTTTAATATTGGGAGCTCCAGATATAGCTTTT
CCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTAGGTTATTGCTTCTTTTATCTTCAGCT
GCTGTGGAAGGAGGTGTTGGGACTGGTTGAACAGTGTATCCTCCTTTAGCTGGAAATTTAGC
ACATGCTGGTGGTTCAGTTGATTTAGCTATTTTTTCACTGCATTTAGCTGGTGCATCTTCTATTT
TAGGGGCTGTTAATTTTATTACTACTGTAATTAATATACGTTGATGTGGTATACAATTTGAGCG
GTTGCCGTTGTTTGTATGATCTGTGAAAATTACTGCTATTTTGCTTTTACTGTCTTTACCAGTGT
TAGCAGGGGCAATTACTATATTATTAACTGATCGAAATTTTAATACATCTTTTTTCGATCCTGCC
GGTGGTGGTGATCCTATTTTATACCAACATTTATTTTGATTTTTTGGTCACCCTGAAAGTTTAAA

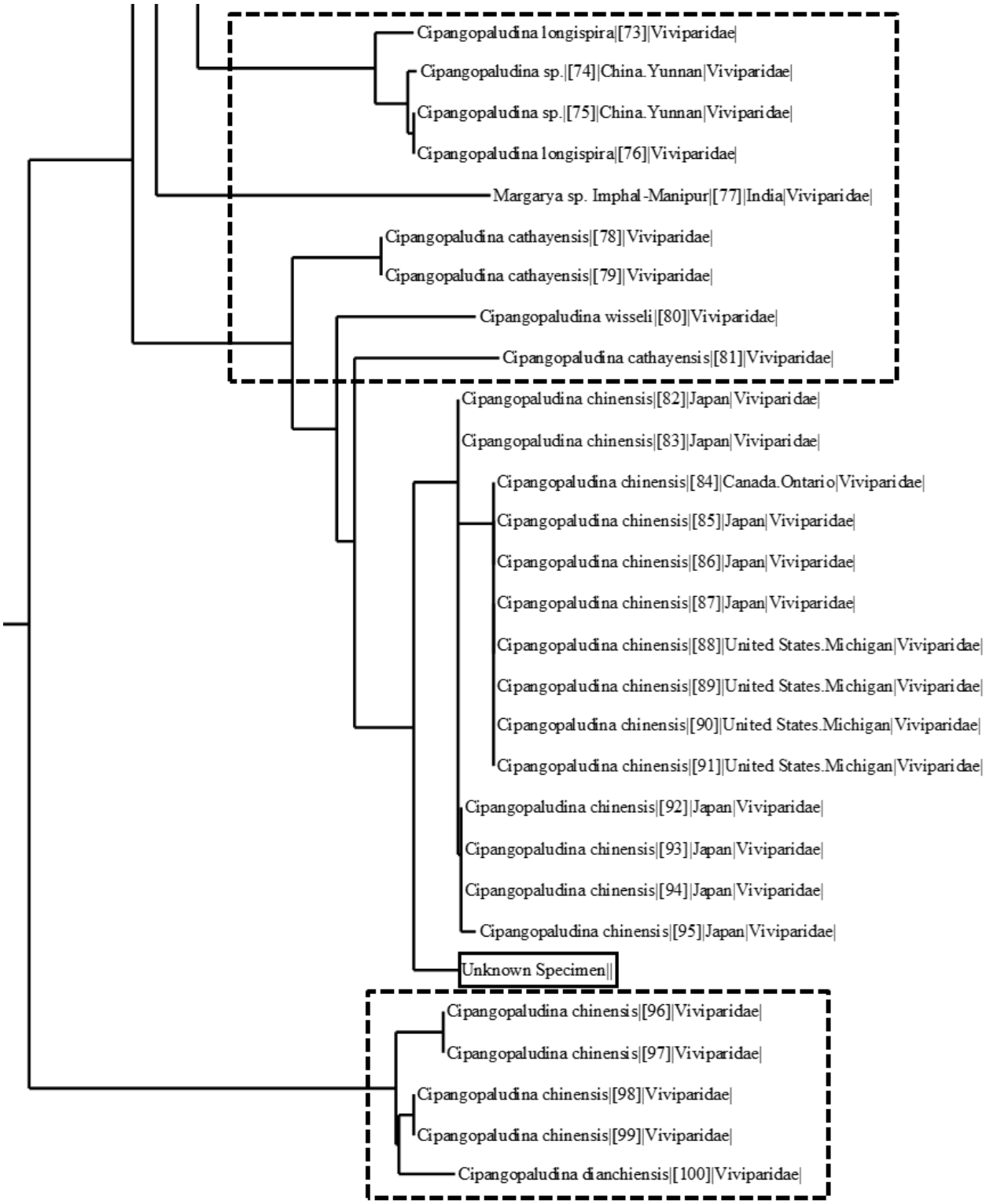
Appendix 1b: Percent Identity Matrix - created by Clustal2.1

Specimen ID	2	OL4	2A	M1	M4	M2	F	M	BL	6	1	4	M3	5	M5
1: 2	100.00	97.32	98.52	97.72	97.88	98.29	97.87	98.14	97.72	98.33	98.64	98.64	97.39	97.83	97.56
2: OL4	97.32	100.00	98.69	98.71	98.71	98.16	97.60	98.65	98.71	98.34	98.34	98.49	98.34	98.53	98.71
3: 2A	98.52	98.69	100.00	99.17	99.38	100.00	99.55	99.12	99.17	99.13	99.55	99.55	98.76	98.91	98.96
4: M1	97.72	98.71	99.17	100.00	99.71	99.51	98.94	99.49	99.28	99.52	99.51	99.49	99.28	99.36	99.57
5: M4	97.88	98.71	99.38	99.71	100.00	99.51	98.94	99.49	99.15	99.36	99.51	99.49	99.29	99.20	99.58
6: M2	98.29	98.16	100.00	99.51	99.51	100.00	98.93	99.48	99.51	99.01	99.18	99.16	99.18	98.85	99.18
7: F	97.87	97.60	99.55	98.94	98.94	98.93	100.00	99.11	98.94	98.76	98.94	98.94	98.58	98.58	98.76
8: M	98.14	98.65	99.12	99.49	99.49	99.48	99.11	100.00	99.49	99.32	99.83	99.83	99.15	99.15	99.32
9: BL	97.72	98.71	99.17	99.28	99.15	99.51	98.94	99.49	100.00	99.52	99.03	99.49	98.71	99.36	99.29
10: 6	98.33	98.34	99.13	99.52	99.36	99.01	98.76	99.32	99.52	100.00	99.84	99.66	99.20	99.20	99.20
11: 1	98.64	98.34	99.55	99.51	99.51	99.18	98.94	99.83	99.03	99.84	100.00	99.83	99.03	99.02	99.19
12: 4	98.64	98.49	99.55	99.49	99.49	99.16	98.94	99.83	99.49	99.66	99.83	100.00	99.16	99.33	99.49
13: M3	97.39	98.34	98.76	99.28	99.29	99.18	98.58	99.15	98.71	99.20	99.03	99.16	100.00	99.36	99.42
14: 5	97.83	98.53	98.91	99.36	99.20	98.85	98.58	99.15	99.36	99.20	99.02	99.33	99.36	100.00	99.68
15: M5	97.56	98.71	98.96	99.57	99.58	99.18	98.76	99.32	99.29	99.20	99.19	99.49	99.42	99.68	100.00

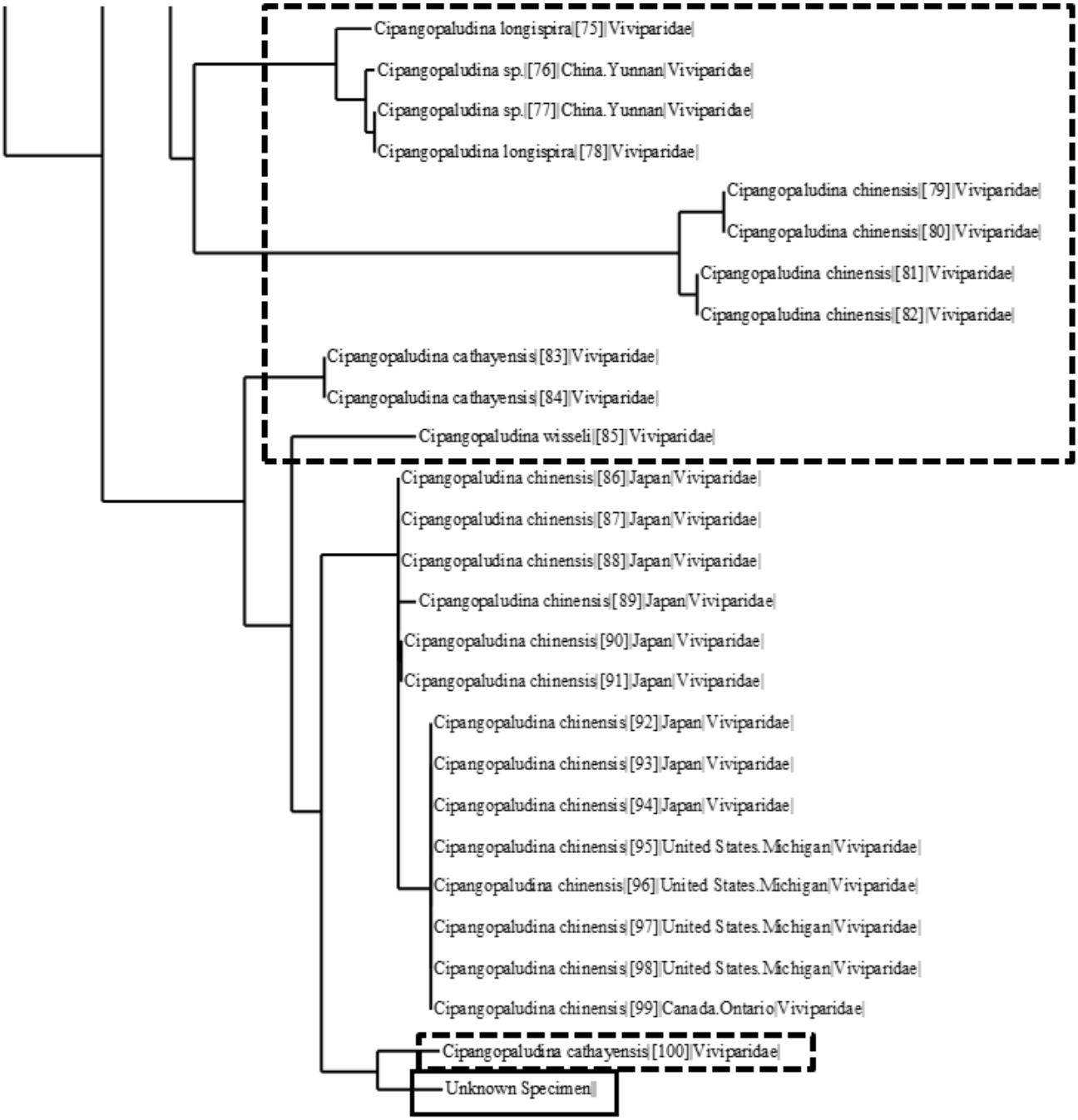
Appendix 2: Phylogenetic trees drawn by BOLD

Example phylogenetic trees for specimens from the different sampling sites are shown. Only the relevant sections containing the specimen and closely related species are shown as full trees cover two pages. Full trees and all other examples can be viewed upon request. Unknown specimens in each tree are outlined with a solid black line, sequences from Genbank are outlined by a dashed line, and all other sequences are from BOLD.

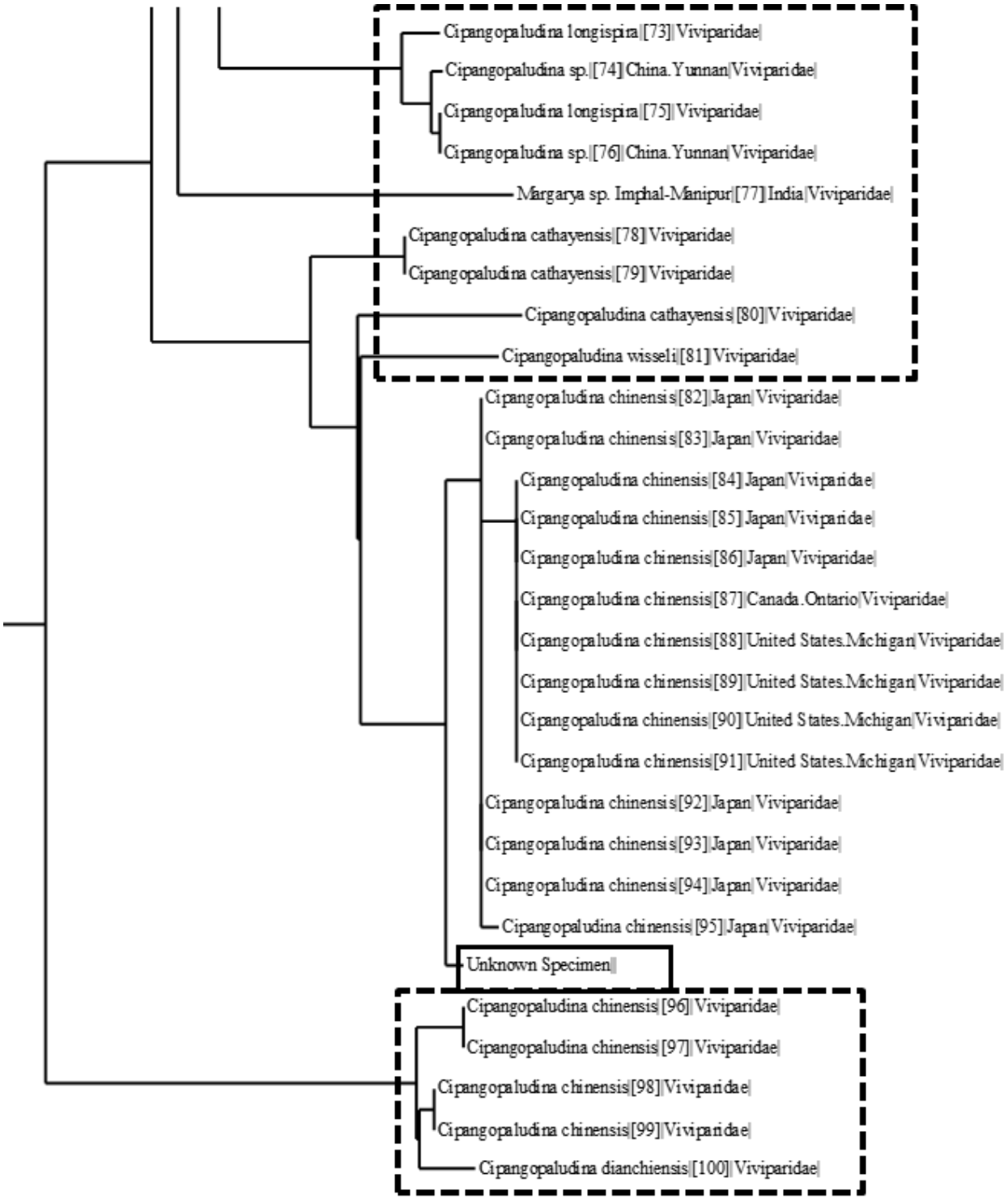
1 Pevensey


Cipangopaludina longispira|[73]|Viviparidae|
Cipangopaludina sp.|[74]|China.Yunnan|Viviparidae|
Cipangopaludina sp.|[75]|China.Yunnan|Viviparidae|
Cipangopaludina longispira|[76]|Viviparidae|
Margarya sp. Imphal-Manipur|[77]|India|Viviparidae|
Cipangopaludina cathayensis|[78]|Viviparidae|
Cipangopaludina cathayensis|[79]|Viviparidae|
Cipangopaludina wisseli|[80]|Viviparidae|
Cipangopaludina cathayensis|[81]|Viviparidae|
Cipangopaludina chinensis|[82]|Japan|Viviparidae|
Cipangopaludina chinensis|[83]|Japan|Viviparidae|
Cipangopaludina chinensis|[84]|Canada.Ontario|Viviparidae|
Cipangopaludina chinensis|[85]|Japan|Viviparidae|
Cipangopaludina chinensis|[86]|Japan|Viviparidae|
Cipangopaludina chinensis|[87]|Japan|Viviparidae|
Cipangopaludina chinensis|[88]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[89]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[90]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[91]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[92]|Japan|Viviparidae|
Cipangopaludina chinensis|[93]|Japan|Viviparidae|
Cipangopaludina chinensis|[94]|Japan|Viviparidae|
Cipangopaludina chinensis|[95]|Japan|Viviparidae|
Unknown Specimen||
Cipangopaludina chinensis|[96]|Viviparidae|
Cipangopaludina chinensis|[97]|Viviparidae|
Cipangopaludina chinensis|[98]|Viviparidae|
Cipangopaludina chinensis|[99]|Viviparidae|
Cipangopaludina dianchiensis|[100]|Viviparidae|

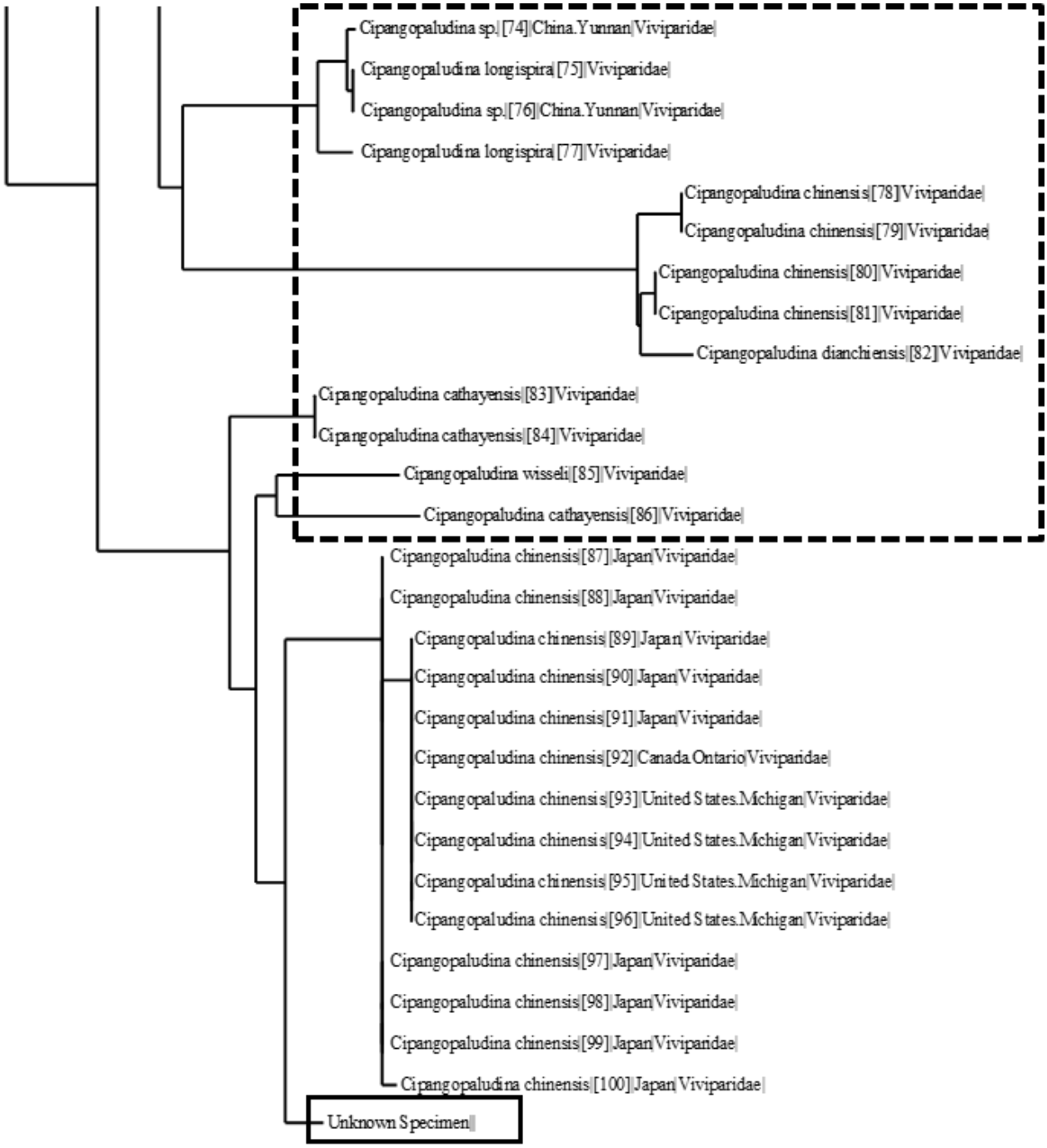
2a Pevensey


Cipangopaludina longispira|[75]|Viviparidae|
Cipangopaludina sp.|[76]|China.Yunnan|Viviparidae|
Cipangopaludina sp.|[77]|China.Yunnan|Viviparidae|
Cipangopaludina longispira|[78]|Viviparidae|
Cipangopaludina chinensis|[79]|Viviparidae|
Cipangopaludina chinensis|[80]|Viviparidae|
Cipangopaludina chinensis|[81]|Viviparidae|
Cipangopaludina chinensis|[82]|Viviparidae|
Cipangopaludina cathayensis|[83]|Viviparidae|
Cipangopaludina cathayensis|[84]|Viviparidae|
Cipangopaludina wisseli|[85]|Viviparidae|
Cipangopaludina chinensis|[86]|Japan|Viviparidae|
Cipangopaludina chinensis|[87]|Japan|Viviparidae|
Cipangopaludina chinensis|[88]|Japan|Viviparidae|
Cipangopaludina chinensis|[89]|Japan|Viviparidae|
Cipangopaludina chinensis|[90]|Japan|Viviparidae|
Cipangopaludina chinensis|[91]|Japan|Viviparidae|
Cipangopaludina chinensis|[92]|Japan|Viviparidae|
Cipangopaludina chinensis|[93]|Japan|Viviparidae|
Cipangopaludina chinensis|[94]|Japan|Viviparidae|
Cipangopaludina chinensis|[95]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[96]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[97]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[98]|United States.Michigan|Viviparidae|
Cipangopaludina chinensis|[99]|Canada.Ontario|Viviparidae|
Cipangopaludina cathayensis|[100]|Viviparidae|
Unknown Specimen|

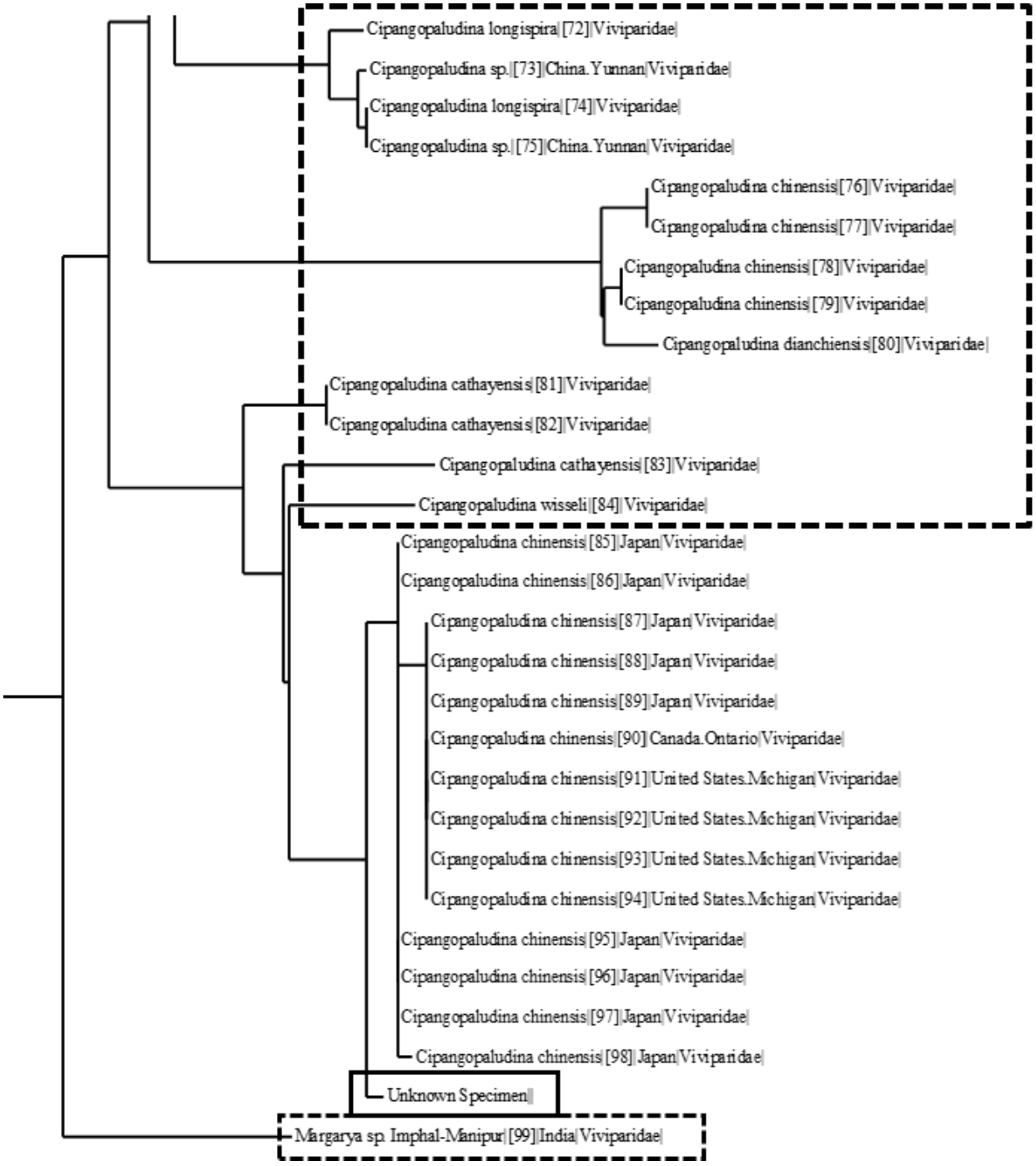
BL Southampton



OL4 Southampton



M1 Southampton



Appendix 3: Sequence accession numbers used for MEGA tree drawing

Species	Accession Numbers Or BOLD ID Numbers
Cipangopaludina chinensis	ANNMO023-18 ANNMO024-18 ANNMO346-19 ANNMO347-19 BFMN099-18 BFMN100-18 BFMN103-18 BFMN104-18 BFMN105-18 BFMN106-18 BFMN108-18 BFMN109-18 BFMN110-18 NC_035734.1 SWOJP327-18
Cipangopaludnia cathayensis	NC_025577.1
Cipangopaludina ussuriensis	MN997923
Cipangopaludina dianchiensis	GU198781
Bellamya aeruginosa	KP150617
Bellamya angularis	KF535546
Bellamya dispiralis	KF535501
Bellamya purificata	KF535374
Bellamya quadrata	KF535469
Viviparus viviparus	KY574013
Viviparus conectus	FJ405835
Margarya melanioides	GU198765
Margarya monodi	GU198796

www.gov.uk/natural-england