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First report of the pygmy slipper lobster *Biarctus sordidus* (Crustacea, Decapoda) in the Red Sea following the finding of its phyllosoma larva

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Abstract

The advent of integrative taxonomy in plankton research, employing molecular and morphology-based identification, promotes the discovery of new biodiversity records, especially of larval stages. The slipper lobster family Scyllaridae consists of planktonic phyllosoma larvae, persisting weeks to many months in the water column. High interspecific larval similarities and inconsistent delineation of stages have hindered the identification of scyllarid phyllosomata to the species level using morphological characteristics. Here we report the first record of the pygmy slipper lobster, *Biarctus sordidus*, in the Red Sea following the finding of its phyllosoma larva, extending its known distribution from the Persian Gulf to Australia and southern China. We identified the phyllosoma collected from the Northern Gulf of Aqaba as *B. sordidus* using the mitochondrial 16S and 18S rRNA genes, and described its morphology to determine the larval stage. We further discuss the potential factors contributing to the delayed detection of this species.

Introduction

Slipper lobsters of the family Scyllaridae are widespread in shallow, warm temperate and tropical habitats. Their life cycle includes several planktonic phyllosoma stages, lasting weeks to months, and a final phyllosoma instar that metamorphoses into nisto, a nektonic postlarva that transforms to the benthic adult (Lavalli and Spanier, 2007). Among scyllarids, the pygmy slipper lobster, *Biarctus sordidus* (Stimpson, 1860), is one of the smallest species. Its distribution spans across the Indian Ocean, from Australia, Indonesia, Borneo, Philippines, Singapore, Malaysia, Thailand, South China and the Persian Gulf, at shallow depths of 2.7 to 73 m (Holthuis, 2002). Phyllosoma larvae of *B. sordidus* were reported from Australia (Barnett, 1989; Mcwilliam *et al.*, 1995), Japan (Sekiguchi, 1997), Java (Tampi and George, 1975), and India (George and Thomas, 1997; Sankolli and Shenoy, 1973). However, neither adults nor larvae of *B. sordidus* were previously recorded in the Red Sea.

Identifying scyllarid phyllosomata to species based on their morphology is difficult due to incomplete descriptions or assignments of developmental stages, as well as disagreements among authors regarding the distinguishing characteristics of these stages (Pagliarino *et al.*, 2013). Employing integrative taxonomy by combining DNA barcoding and morphological characterization is therefore essential for establishing the phyllosoma larval stages of scyllarid species (Guy-Haim *et al.*, 2024). Here we used integrative taxonomy to describe an early stage phyllosoma of *B. sordidus*, recorded for the first time in the Red Sea.

Materials and methods

A plankton sample was collected in 6 March 2024 at 6:00 AM using a plankton net (200-µm mesh size, 50 cm opening diameter, Aquatic Research Instruments, USA), towed horizontally for 10 min at a depth of 0.5–1 m in Station A, located at the northern Gulf of Aqaba, Eilat, bottom depth ca. 700 m (29°28′N, 34°55′E, Figure 1A). Fieldwork was conducted under INPA permit no. 2024-43522.

The plankton sample was examined under a stereomicroscope (SZX16, Olympus, Japan). A phyllosoma larva was identified morphologically and the developmental stage was determined following Sankolli and Shenoy (1973) and Ritz (1977). One pereiopod was cut for molecular identification, and the specimen was stored in 70% ethanol and deposited in the zooplankton collection, the National Natural History Collections, Hebrew University of Jerusalem (NNHC, HUJI).

Total genomic DNA was extracted from the pereiopod using the InviSorb Spin Tissue Mini Kit (Invitek Diagnostics, Germany) according to the manufacturer's specifications. Following the DNA extraction, the 18S rRNA gene was amplified using the primers 18S-3F and 18S-9R

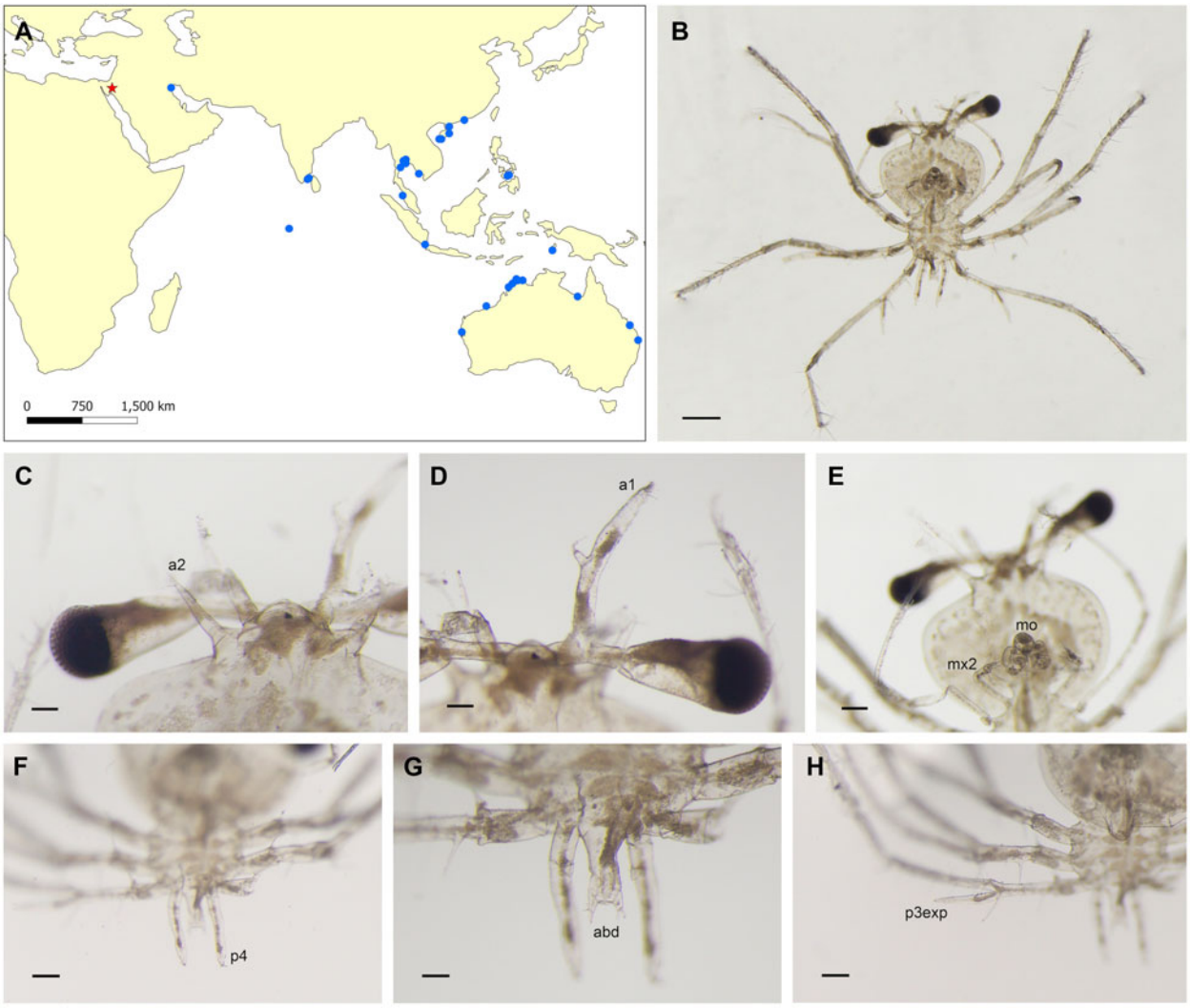

Figure 1. A. The distribution map of *Biarctus sordidus*. Occurrences are presented in blue circles and the new record in a red star. Occurrences were downloaded from https://www.gbif.org/ and OBIS https://obis.org/ on 17 April 2024. Additional occurrences were added from Holthuis (2002). **B–H.** *B. sordidus* phyllosoma stage III/IV. **B**. ventral view. **C.** Antenna (a2). **D.** Antennule (a1). **E.** Mouth (mo) and maxillipeds. **F**. Pereiopod 4 (p4). **G**. Abdomen (abd). **H**. Exopod of pereiopod 3 (p3exp). Scale bars: B – 500 µm, C – 100 µm, D – 100 µm, E – 500 µm, F – 200 µm, G – 100 µm, H – 100 µm.

following Giribet *et al.* (1999) and the mitochondrial 16S gene was amplified using the primers 16Sar-16Sbr following Palumbi (1996). Reaction conditions were as follows: 94°C for 2 min, followed by 34 cycles of 94°C for 15 s, 49°C for 30 s, and 72°C for 1 min, and a final elongation step of 72°C for 7 min. Obtained PCR products were purified and sequenced by Hylabs (Rehovot, Israel).

A total of twenty-one 18S rRNA sequences of Scyllaridae were analysed, including one sequence of the phyllosoma of *B. sordidus* obtained in this study, one sequence of adult *B. sordidus* from Thailand (JN701622), one sequence of *B. vitiensis* from Guam (JN701621), and additional 18 scyllarid sequences obtained from GenBank (accession numbers and collection locality are indicated in Figure 2). *Ibacus peronii* and *I. chacei* were used as an outgroup.

A total of fifteen 16S mtDNA sequences of Scyllaridae were analysed, including one sequence of the phyllosoma of *B. sordidus* obtained in this study, two sequences of adult *B. sordidus* from Thailand (JN701710) and Australia (AY583888), two sequences of *B. pumilus* from South Africa (ON960177- ON960178), one sequence of *B. vitiensis* from Guam (JN701709), and additional nine scyllarid sequences obtained from GenBank (accession numbers and collection locality are indicated in Figure 3). *Ibacus chacei* was used as an outgroup.

Sequence alignments were conducted using ClustalW embedded in MEGA v11.0 (Tamura *et al.*, 2021). The best-fitting substitution models were selected according to the Bayesian Information Criterion using Maximum-likelihood (ML) model selection in MEGA. ML analyses were performed using the K2 + G (18S) and HKY + G (16S) models with 1000 bootstrapping replicates each.

Results

Morphological description

Biarctus sordidus (Stimpson, 1860): phyllosoma, stage III (following Sankolli and Shenoy, 1973) / IV (following Ritz, 1977) (Figure 1B).

Dimensions: TL (total length) 2.24 mm; CL (cephalic length) 1.52 mm; CW (cephalic width) 1.49 mm; ThW (thorax width) 0.73 mm; ThL (thorax length) 0.76 mm; A1L (antennular length) 0.63 mm; A2L (antennal length) 0.22 mm; AbdL (abdomen length) 0.21 mm.

Cephalic shield subcircular, twice wider than thorax. Eyestalks segmented. Antennule unsegmented with inner flagellum as a small bud. Right antennule damaged. Antenna short uniramous with small seta on either side of tip. Pereiopod 3 with unsegmented setose exopod. Pereiopod 4 more than twice longer than abdomen. Uropods not visible.

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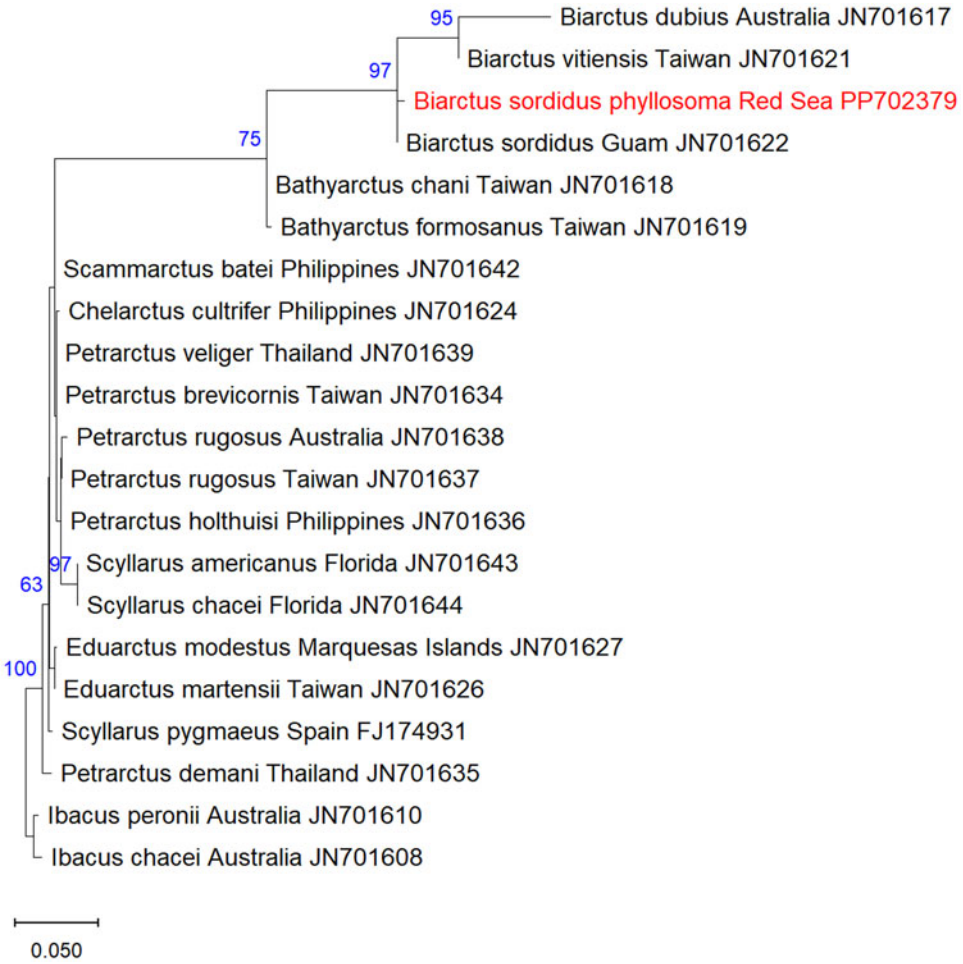

Figure 2. Maximum-Likelihood phylogenetic tree of *Biarctus sordidus* based on the 18S rRNA gene, using the K2 + G substitution model. The outgroups *Ibacus peronii* and *I. chacei* were used as a root node. The numbers in blue indicate the percentage of ML bootstrap support (1000 replicates) for nodes that received at least 60% support. The scale bar denotes the estimated number of nucleotide substitutions per site.

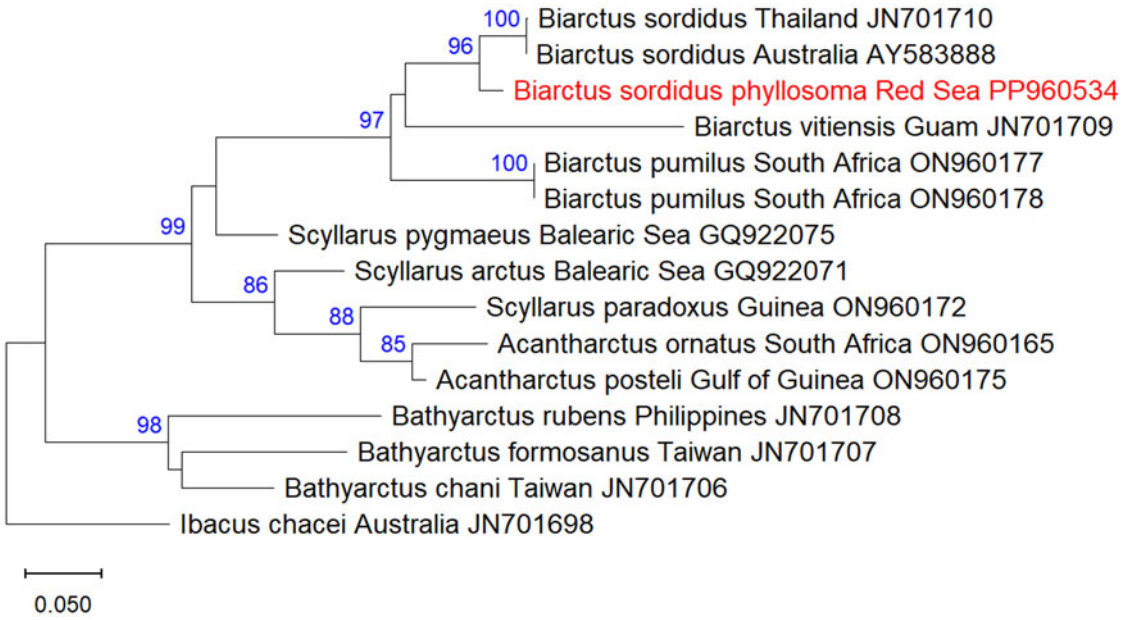

Figure 3. Maximum-Likelihood phylogenetic tree of *Biarctus sordidus* based on the mitochondrial 16S gene, using the HKY + G substitution model. The outgroup *Ibacus chacei* was used as a root node. The numbers in blue indicate the percentage of ML bootstrap support (1000 replicates) for nodes that received at least 60% support. The scale bar denotes the estimated number of nucleotide substitutions per site.

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Molecular identification

A DNA fragment of 577 bp of the 18S rRNA gene was sequenced from the phyllosoma larva of *B. sordidus* and assembled from forward and reverse sequences. The sequences were deposited in NCBI GenBank under the accession numbers PP702379 (18S) and PP960534 (16S). NCBI blastn yielded 99.61% identity of the 18S sequence to *B. sordidus* from Guam, and 96.33% identity of the 16S sequence to *B. sordidus* from Thailand. Maximum likelihood analysis of 18S Scyllaridae sequences obtained from GenBank showed that the phyllosoma from the Red Sea is clustered together with *B. sordidus* within the *Biarctus* clade, with high bootstrap support.

Discussion

The Red Sea is home to seven scyllarid species: the clamkiller slipper lobster, *Scyllarides tridacnophaga,* the Aesop slipper lobster, *S. haanii*, the dark-spot locust lobster, *Gibbularctus gibberosus*, the flathead lobster, *Thenus orientalis,* the hunchback locust lobster *Petrarctus rugosus, Eduarctus lewinsohni*, and *Biarctus pumilus* (Holthuis, 1968; Holthuis, 2002). Here we report an eighth scyllarid in the Red Sea, *Biarctus sordidus*, following the finding of its phyllosoma larva in the northern tip of the Gulf of Aqaba, Eilat.

Using laboratory-hatched eggs of adults collected from Moreton Bay, southeast Queensland, Australia, Ritz (1977) described the first phyllosoma stage of *B. sordidus*, and based on plankton samples he provided keys to its eight phyllosoma stages. Sankolli and Shenoy (1973) described the first six phyllosoma stages of laboratory hatched *B. sordidus* collected from western India, stating that 'each phyllosoma stage (instar) took, on an average, five days to moult to the next'. Thus, it can be estimated that the larval duration of *B. sordidus* is approximately 40 days. We found the stage III/IV phyllosoma of *B. sordidus* in the beginning of March in the offshore waters of the northern Gulf of Aqaba. We can therefore hypothesize that a potential recruitment of this species in the Red Sea may take place during April.

Similar to its congener *B. pumilus*, *B. sordidus* inhabits shallow hard sandy bottoms within its distributional range. The location and shape of the cardiac and gastric teeth, minute morphological features, differentiate between these sister taxa (Holthuis, 2002). Thus, it is plausible that former biodiversity surveys in the Red Sea have confused *B. sordidus* with *B. pumilus*. Another reason for the late detection of *B. sordidus* in the Red Sea might be a recent introduction by ship ballast, as was previously hypothesized to explain the introduction of the Caribbean spiny lobster *Panulirus argus* to Cape Verde Archipelago (Freitas and Castro, 2005). Cargo ships that arrived at the Port of Eilat in Israel and the Port of Aqaba in Jordan, both located at the Northern Gulf of Aqaba, could have been the vector of such an introduction. Nonetheless, there is no former evidence for ballast-mediated introductions of phyllosoma larvae. Further studies are needed to unveil whether the pygmy slipper lobster has established populations in the Red Sea.

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Author contributions. T.G-H. has conceived the study, analyzed the data, interpreted the findings and written the article. A.I. and A.R.M. have analyzed the data. V.F. has collected the data. E.S. has assisted in the interpretation of the findings. All coauthors contributed to the writing of the article.

Competing interests. The authors declare no competing interests.

Data Availability Statement. The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/, and can be accessed with accession numbers PP702379.1 and PP960534.1.

References

Barnett B (1989) Final-stage phylosoma larvae of *Scyllarus* species (Crustacea: Decapoda: Scyllaridae) from shelf waters of the Great Barrier Reef. *Invertebrate Systematics* **3**, 123–134.

Freitas R and Castro M (2005) Occurrence of *Panulirus argus* (Latreille, 1804)(Decapoda, Palinuridae) in the northwest islands of the Cape Verde archipelago (central-east Atlantic). *Crustaceana* **78**, 1191–1201.

George RM and Thomas P (1997) The early stage of *Scyllarus sordidus* (Stimpson) from the inshore plankton off Vizhinjam together with a note on their distribution. *Journal of the Marine Biological Association of India* **38**, 74–79.

Giribet G, Carranza S, Riutort M, Baguñà J and Ribera C (1999) Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **354**, 215–222.

Guy-Haim T, Iakovleva A, Ermak K, Spanier E and Morov AR (2024) First record of the phyllosoma larva of the pygmy locust lobster *Scyllarus pygmaeus* (Crustacea, Decapoda) in the Eastern Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* **104**, e49.

Holthuis LB (1968) The palinuridae and scyllaridae of the Red Sea. *The second Israel South Red Sea Expedition, 1965* **42**, 281–301.

Holthuis LB (2002) The Indo-Pacific scyllarine lobsters (Crustacea, Decapoda, Scyllaridae). *Zoosystema* **24**, 499–683.

Lavalli KL and Spanier E (2007) *The Biology and Fisheries of the Slipper Lobster*. Boca Raton, FL: CRC Press.

Mcwilliam PS, Phillips BF and Kelly S (1995) Phyllosoma Larvae of *Scyllarus* Species (Decapoda, Scyllaridae) from the shelf waters of Australia. *Crustaceana* **68**, 537–566.

Pagliarino E, Massi D, Canali E, Costa C, Pessani D and Bianchini M (2013) Findings of phyllosoma larvae and nistos of the family Scyllaridae (Crustacea, Decapoda) in the Southern Mediterranean Sea. *The Open Marine Biology Journal* **7**, 8–13.

Palumbi S (1996) Nucleic acids II: the polymerase chain reaction In: Molecular systematics by Hillis D, Moritz C, Mable BK, eds, Sunderland, MA. Sinauer Associates Inc.

Ritz D (1977) The larval stages of *Scyllarus demani* Holthuis, with notes on the larvae of *S. sordidus* (Stimpson) and *S. timidus* Holthuis (Decapoda, Palinuridea). *Crustaceana* **32**, 229–240.

Sankolli K and Shenoy S (1973) On the laboratory hatched six phyllosoma stages of Scyllarus sordidus (Stim). *Journal of the Marine Biological Association of India* **15**, 218–226.

Sekiguchi H (1997) Larval recruitment processes of Japanese spiny lobsters: a hypothesis. *Proceedings of the Bulletin of Marine Science* **1997**, 43–55.

Stimpson W (1860) Prodromus descriptionis animalium evertebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem, a Republic Federata missa, Cadwaladore Ringgold et Johanne Rodgers Ducibus, observavit et descripsit. Pars VIII, Crustacea Macrura. *Proceedings of the Academy of Natural Sciences of Philadelphia* **12**, 31–40.

Tampi P and George M (1975) Phyllosoma larvae in the IIOE (1960–65) collections-systematics. *Mahasagar* **17**, 56–107.

Tamura K, Stecher G and Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* **38**, 3022–3027.

https://doi.org/10.1017/S0025315424001085 Published online by Cambridge University Press