学位論文

Evolutionary process of deep-sea fishes in the subfamily Lycodinae in the northwestern Pacific

（北西太平洋におけるマユガジ亜科深海魚の進化過程）

平成 25 年 12 月 博士（理学）申請

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Abstract

The present patterns of biodiversity are products of geological and biological factors that have been continuously acting upon them. The Pleistocene glacial cycles have strongly affected the range and the population size of marine taxa. Above all, the marginal seas of the northwestern Pacific Ocean were struck by the severe climate changes. Recently, it has been uncovered that fluctuations of sea-level and water temperature during the mid- to late Pleistocene had strongly affected the spatial distribution and the population size of coastal marine and anadromous species in the Sea of Japan, while their impacts on the offshore, deep-sea organisms remain elusive.

I investigated phylogenetic relationships, the present-day distribution, the population history and the speciation process of deep-sea fishes of the subfamily Lycodinae in this study, to test the hypothesis that the past climatic events had contributed to the present deep-sea biodiversity. The fishes of the subfamily Lycodinae lack planktonic eggs and larval phases in their early life stages and therefore, they have limited larval dispersal ability. In addition, they mainly inhabit the continental shelves and slopes in the northwestern Pacific Ocean, where the drastic climate changes have repeatedly induced the isolation and recombination of deep-sea habitats. These ecological characters could have contributed to the fragmentation of deep-sea habitats in the marginal seas of the northwestern Pacific Ocean during the climatic oscillations of the mid- to late-Pleistocene.

I investigated phylogenetic relationships within the subfamily Lycodinae based on nucleotide sequences of four mitochondrial (12S rRNA, 16S rRNA, cytochrome *b*, cytochrome oxidase *c* subunit I) genes and the nuclear Rhodopsin retrogene from 23 zoarcid species in the Chapter 1. The results by the maximum-likelihood and the Bayesian inference were basically concordant and strongly supported the monophyly of each subfamily (Gymnelinae, Zoarcinae, Lycodinae). However, the relationships between species in Lycodinae were different from previous morphological hypothesis, suggesting polyphyly of the *Lycodes*, which is the largest genus in Lycodinae encompassing about 60 species. A

parsimony-based reconstruction of ancestral character infer the acquisition of the submental crest, the cartilaginous organ under the jaw, which characterizes the genus *Lycodes*, possibly allowed several lineages of Lycodinae to diversify in the deep-sea floor, independently.

In the Chapter 2, I investigated the geographic distribution, bathymetric distribution and the species composition of the species of Lycodinae. In addition, the environmental factors that provide such patterns of diversity in the southwestern Sea of Japan were examined. I obtained a total of 7,518 specimens of six species from 141 sites. Each of them showed a highly specific pattern of geographic distribution. The nMDS (non-metric multi-dimensional scaling) analyses showed the spatial heterogeneity in the species composition of *Lycodes* assemblages and the DistLM (Distance-based liner model) suggested that the abiotic environmental variables such as depth and water temperature contribute to such spatial pattern.

I examined the mitochondrial control region and cytochrome *b* gene sequences of *Lycodes matsubarai*, which inhabits the Sea of Japan and the Sea of Okhotsk to elucidate the effects of the last glacial period on the populations in these two regions in the Chapter 3. My results show clear genetic differentiation between the two populations. The two populations may have diverged during the LGP, probably because of the vicariance due to the drastic sea level change. The population in the Sea of Okhotsk had been larger than that in the Sea of Japan, but it suddenly decreased after the LGP, possibly owing to interspecific competition. The Sea of Japan population, by contrast, expanded after the LGP, coincident with high levels of oxygenation in the deep-sea areas. These results elucidate the regional-scale impacts of climate changes on deep-sea organisms.

In the Chapter 4, I performed the population genetic study on the pair of sibling species, *Lycodes japonicus* and *L. ocellatus*, distributed in the Sea of Japan and the northwestern Pacific Ocean, respectively, to evaluate the role of a vicariance event during the mid- to late-Pleistocene climatic oscillation. Nucleotide sequences of two mitochondrial genes, viz. cytochrome oxidase subunit I and cytochrome *b*, reconfirmed that the two species are genetically distinct from each other. The two species are estimated to have diverged 0.2–0.8

million years ago, which suggests that they were isolated from each other by the sea-level change during the mid- to late Pleistocene. The population size of *L. japonicus* was consistently smaller than that of *L. ocellatus* but is still expanding, which may indicate that *L. japonicus* is recovering from a population reduction as a result of drastic environmental changes during the LGP in the Sea of Japan. Results in this study show the clear evidences of the glacial impacts that have strongly influenced the spatial distribution, the population size and the species diversity of the subfamily Lycodinae. The six Lycodinae species in the southwestern Sea of Japan showed localized distribution even within the narrow sea area (Chapter 2), which may have contributed the allopatric speciation process suggested in the Chapters 1 and 4. The drastic changes in the population size of *L. matsubarai* are also indicative of a regional variation in responses to drastic environmental changes during the past glacial periods. The present results, which show the impacts of glacial climate changes in the marginal seas of the northwestern Pacific, might help explain the deep-sea biodiversity in these seas.

Contents

General introduction

1.1. Historical process that have shaped the present biodiversity

The present patterns of biodiversity are products of geological and biological factors that have been continuously acting upon them. The mechanisms that have shaped the present biodiversity are the central subject in the evolutionary biology. Marine biodiversity have been fluctuated several times throughout the long history of the earth. Above all, widespread and rapid decreases in the number of species have occurred at the boundary of the Ordoviician-Silurian (446 million years ago: Mya), Frasnian-Famennian (371 Mya), the Permian-Triassic (251 Mya), the Triassic-Jurassic (200 Mya) and Cretaceous-Paleogene boundaries (65 Mya: McElwain and Punyasena 2007). These events known as "mass extinctions" have reset the evolutionary process of marine taxa, and the marine ecosystems have been replaced by the new ones (Gould 1994). Although there is no consensus at present on the causes of these mass extinction events, it is generally accepted that abiotic environmental changes have caused these events. These major events are worthy of note because of their amplitude, while the most important event that has shaped the present patterns of marine faunal and floral diversity is the glacial episodes in the Quaternary (2.6 Mya to present: Avise 2000; Provan and Bennett 2008).

1.2. The Quaternary ice ages and their impacts on marine organisms

Global climate has greatly fluctuated during the past three million years (My), which have led to the recent major ice ages. From the beginning of the Quaternary (about 2.6 Mya) when the Arctic ice cap became established, the ice sheets advanced and receded in a roughly 41,000-year cycle until 0.9 Mya; thereafter, the duration of each cycle became 100,000 years, and the changes in climate became increasingly drastic (Williams et al. 1998). The severe climatic changes altered distributions of terrestrial taxa. Advancing glacial ices forced species to survive

in refugial areas, from which they expanded ranges when the ice receded (Hewitt 2000).

The drastic changes in water temperature during the Pleistocene have strongly affected the spatial distribution and the diversity of coastal marine taxa, but with considerable local variation. In the Pacific Ocean, the extinction rate of coastal marine gastropods did not increased during the Pleistocene climate changes, but the extensive species range shifts were induced (Roy et al. 1995). In the western Atlantic Ocean, early Pleistocene extinction rates were estimated to be about twice as large as those in the eastern Pacific Ocean. Instead, the speciation was suggested to have accelerated and the present diversity appears to be similar to the levels before the glaciation (Roy et al. 1998).

Knowledge about marine glacial refugia has been increasing in the last decade (Fig. I). In Europe, it has been reported that various taxa including seaweeds (*Palmaria palmate*, *Ascophyllum nodosum*, *Fucus serratus*) and the thornback ray (*Raja clavata*) had persisted during the last glacial maximum (LGM) around the English Channel and that postglacial recolonization of the eastern North Atlantic took place from this refugial area (Coyer et al. 2003; Provan et al. 2005; Hoarau et al. 2007; Chevolot et al. 2006). The three-spined stickleback, *Gasterosteus aculeatus*, was suggested to have survived in the Mediterranean region (Mäkinen and Merilä 2008). In the northeastern Pacific Ocean, two scenarios have been presented: 1) the southern refugium hypothesis and 2) the range persistence hypothesis. Both of the two theories have been supported by various empirical studies, which also led us to understand the influences of the ecological characteristics of each species such as the dispersal ability (Hickerson and Cunningham 2005) and habitat depth (Marko 2004).

The population size of coastal ￥species has also been affected by the Pleistocene glacial cycles. Grant and Bowen (1998) referred to the link between climatic regime shift, oceanographic conditions and the population history of marine organisms, to explain the shallow population histories of ‘old’ lineages such as sardines and anchovies. Subsequently, Lecomte et al. (2004) dated the population expansions of the East Pacific anchovy, *Engraulis mordax*, and the South American pilchard, *Sardinops sagax*, to be at about 282,000 and 241,000 years ago, respectively, possibly during the Kansan glacial maximum that largely affected the

Northern Hemisphere. Most of coastal marine fishes have been suggested to have experienced recent population expansions, which were likely triggered by the drastic climate changes (Avise 2000).

Few studies have elucidated the influences of the glacial impacts on deep-sea species. Furthermore, the previous studies often failed in detecting the factors controlling the population size of deep-sea fishes because the traces of population history were always hidden under a cover of the genetic homogeneity over wide areas. Most species subject to these studies are widely distributed and some species have a cosmopolitan distribution (Varela et al. 2012). Such species tend to be genetically homogeneous over its distribution range (White et al. 2011; Takeshima et al. 2011). Varela et al. (2012) suggested that the effective population size of the orange roughy, *Hoplostethus atlanticus*, dramatically changed through the glacial-interglacial cycles, which might be attributable to the changes in the marine environment. Although demographic expansion has also been reported for the alfonso *Beryx decadactylus*, (Friess and Sedberry 2011) and the black scabbardfish *Aphanopus carbo* (Stefanni and Knutsen 2007), the causes of these demographic changes have not been discussed.

1.3. Current knowledge of glacial impacts on the marine organisms in the northwestern Pacific

In the northwestern Pacific, there are several marginal seas, which are semi-enclosed and partially isolated from adjacent parts of the Pacific Ocean by submarine ridges on the sea floor (i.e., the Bering Sea, the Sea of Japan, the Sea of Okhotsk, the East China Sea and the South China Sea: Fig. III). These seas were situated along the world's largest subduction zone in the Western Pacific (Tamaki and Honza 1991) and separate the Asian Continent from the Pacific Ocean. Since they were formed in the late Cenozoic, the marginal seas have characterized the geographical feature of the west Pacific (Wang 1999).

It is evident that the fauna and flora in the marginal seas of the northwestern Pacific Ocean have been heavily influenced by the severe climate changes during the Quaternary ice

ages. During the past, major glaciations, the large volume of ice was accumulated on the Polar ice sheets and the major mountain blocks (i.e., Alps, Andes and Rockis) and sea levels were lowered by approximately 120 m (Fig. II, Miller et al. 2005). These sea-level fluctuations were amplified in the marginal seas and induced drastic environmental changes (Wang 1999; Voris 2000). For example, the Sunda Shelf, the large shelf area between the Indonesian archipelago and Vietnam, was shown to have been widely exposed during the last glacial period, forming a semi-enclosed marginal area in the South China Sea (Hanebuth et al. 2000, Fig. III). In the Bering Sea, the Aleutian basin was partially separated from the North Pacific due to the glaciation around the Aleutian Islands (Mann and Hamilton 1995). Among these sea areas, the Sea of Japan was especially unique as it was suggested to have experienced drastic environmental changes during the last glacial period. The lowered sea-level formed land bridges in four shallow straits which connect the sea with the Pacific Ocean, isolating it from the surrounding seas (Park et al. 2000; Yokoyama et al. 2007, Fig. III). In most parts of the Sea of Japan, the strong density stratification of water columns developed during the LGM, which resulted in fatally anoxic sea-bottom conditions (Itaki et al. 2004). Such environmental changes might have strongly influenced the fauna and flora not only in the Sea of Japan, but also over the northwestern Pacific region.

Little is known about the impacts of glaciations on the marine species in the marginal seas of the northwestern Pacific Ocean. The low sea-level stands produced land bridges in the several parts of the western Pacific Ocean, which induced isolation of marine habitats and drove divergence of populations of coastal marine organisms (Liu et al. 2007; Shen et al. 2011). Population genetic studies have shown the responses to the climate changes in the populations of anadromous fishes such as the ice goby *Leucopsarion petersii* (Kokita and Nohara 2011) and intertidal fishes as the forktongue goby *Chaenogobius annularis* (Hirase et al. 2012). They share history of the vicariance between the Sea of Japan and the Pacific Ocean following the sudden population growth in the Sea of Japan. Kodama et al. (2008) and Kojima et al. (2011) also showed population divergence of the Japan Sea eelpout *Bothrocara hollandi* between the Sea of Japan and neighboring seas, which may be attributed to the vicariance due to the drastic

sea-level changes in the LGM. However, these examples in the northwestern Pacific region do not allow us to infer the general trends in the response to the glacial environmental changes.

1.4. Evolutionary process of the deep-sea fishes in the subfamily Lycodinae

In this thesis, I focus on the deep-sea fishes in the subfamily Lycodinae in the northwestern Pacific. The subfamily Lycodinae, which was first described by Gill (1862), belongs to the family Zoarcidae, which is known as one of the dominant groups of deep-sea demersal fishes in the northern Hemisphere (Randall and Farrel 1997). While the monophyly of the subfamily Lycodinae has been widely accepted (i.e., Anderson 1994), the molecular phylogenetic evidence has not been provided.

The deep-sea fishes in the subfamily Lycodinae have a great potential for elucidation of the impacts of the drastic climate changes during the Pleistocene, on both population dynamics and evolution of the deep-sea organisms in marginal seas of the northwestern Pacific. The species of the Lycodinae lack planktonic egg and larval periods (Matarese et al. 1989; Balanov et al. 2006) and they are, therefore, expected to have limited dispersal ability and gene flow among local populations. Such characters in the life history make them ideal subjects for investigating the evolutionary process and the population history of deep-sea species.

However, there are some sources of confusion in the taxonomy and systematics of the subfamily Lycodinae. For example, the genus *Lycodes*, the largest genus in this subfamily was defined only on the basis of one morphological character and there still remains a possibility that the character is homoplastic. While the molecular phylogenetic evidences also supported this view (Møller and Gravlund 2003), their analyses were based on poor dataset, including only 700 bp in the mitochondrial DNA and the taxon sampling was also incomplete. To elucidate the evolutionary history of the subfamily Lycodinae, thus, these problems should be clarified.

1.5. The aims and scopes of this thesis

In this thesis, I attempt to reveal the impacts of the drastic climate changes during the Pleistocene on the taxa in the marginal seas of the northwestern Pacific, using the deep-sea fishes in the subfamily Lycodinae in the northwestern Pacific. In the Chapter 1, I reconstruct the phylogenetic relationship among species of the subfamily Lycodinae and discuss which factors have contributed to the diversification of these species. The Chapter 2 presents the detailed geographic and bathymetric distributions of the fishes of Lycodinae in the southwestern Sea of Japan. In this chapter, I demonstrate the existence of the heterogeneous regional-scale assemblage structure of the species of Lycodinae, even in the small geographic area in the open ocean. In the Chapter 3, the genetic population structure of *Lycodes matsubarai*, which inhabits the two neighboring sea areas in the northwestern Pacific, was investigated to clarify the influences of climatic changes of the last glacial period on the population history of deep-sea species. I show the climate changes during the last glacial period induced the formation of population structure. The drastic climate changes during the Pleistocene possibly acted upon the speciation process of deep-sea species. In the Chapter 4, I elucidate the speciation process of *Lycodes japonicus* and *Lycodes ocellatus*, a pair of sibling species inhabiting the Sea of Japan and the northwestern Pacific Ocean, respectively. I suggest that the mid- to late-Pleistocene glaciation had played an important role in the speciation of these species. Finally I discuss the impacts of the Quaternary ice ages on the biodiversity in the marginal seas in the northwestern Pacific and in the deep-sea organisms and propose further perspectives in the General discussion.

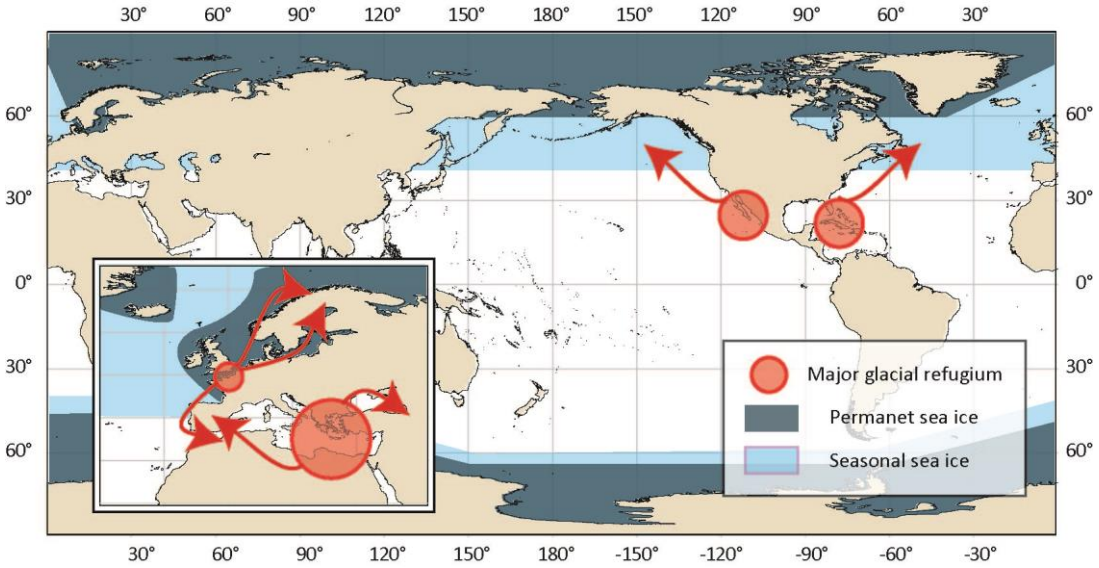

Fig. I The maximum extent of sea ice during the last glacial period (modified from Hewitt 2000). Major Glacial refugia in Europe and the North America, and the post glacial re-colonization pathways are also indicated.

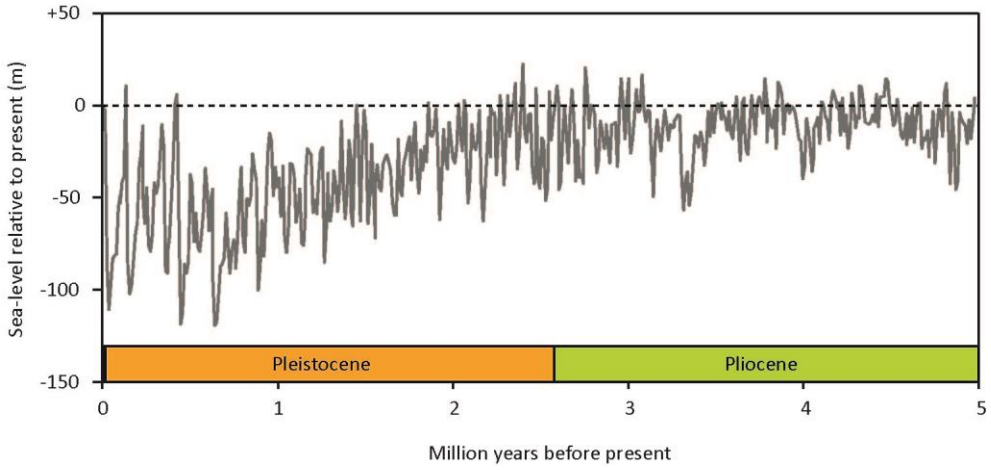

Fig. II Global trends of sea-level change during the last five million years (My), averaged over 5,000 year intervals (modified from Miller et al. 2005). A dashed line indicates the present sea-level.

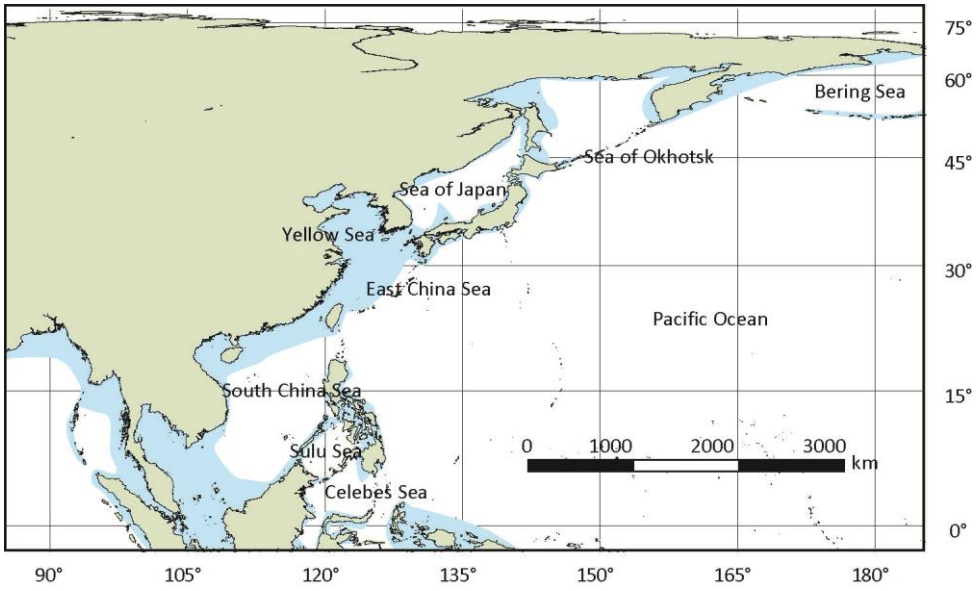

Fig. III A map of the northwestern Pacific Ocean showing the major marginal seas which were influenced by the Quaternary ice ages. Blue areas show the extent of the land areas assuming the lowered sea-level of 120 m.

Chapter 1

Evolutionary history of the subfamily Lycodinae

1.1. Introduction

The subfamily Lycodinae belongs to the family Zoarcidae, a teleost family encompassing more than 230 species in the 46 genera. Anderson (1994) revised the systematics of the family Zoarcidae and established the subfamilies Gymnelinae, Zoarcinae and Lycozoarcinae, together with the 120 species in 32 genera of Lycodinae. The monophyly of the subfamily Lycodinae was supported by three morphological characters: 1) the L-shaped pattern of suborbital bone configuration, 2) the absence of interorbital pores and 3) the oral valve which is laterally constricted. The molecular phylogenetic evidences supporting its monophyly have, however, not been provided at present.

Anderson (1994) classified 64 species into *Lycodes*, the largest genus in the subfamily Lycodinae, based on one autapomorphic character, the submental crest, which was suggested to be cartilage extensions on the lower jaw. Molecular phylogenetic evidences also supported this view (Møller and Gravlund 2003). However, the resolution of the relationships among *Lycodes* species shown by Møller and Gravlund (2003) was very poor, as they used nucleotide sequences of only 714 bp (base pair) from two mitochondrial genes, and taxa around the Japan Archipelago where one third of the *Lycodes* species are distributed (Toyoshima 1985), were not included. In addition, zoarcids were known to be affected by a high degree of homoplasy in morphological characters on both generic and species level (Anderson 1994), therefore, the evolutionary histories of *Lycodes* fishes are still obscure due to the uncertainty of their phylogenetic relationships.

I analyzed the phylogenetic relationships among 22 zoarcid species, three from Zoarcinae, two from Gymnelinae and 17 from Lycodinae. I included taxa around the Japan Archipelago and used molecular phylogenetic methods based on the nucleotide sequence data from four mitochondrial and one nuclear genes. I also assessed the evolutionary history of the

submental crest, which characterizes fishes of the genus *Lycodes*, under the parsimony-based reconstruction of ancestral states. I aim to confirm the monophyly of both the subfamily Lycodinae and the genus *Lycodes*, to elucidate the evolutionary processes of fishes of the Lycodinae in this chapter,.

1.2. Materials and methods

1.2.1. Sample collection and laboratory procedures

Specimens were collected using an otter-trawl during the fisheries surveys conducted by the Fisheries Research Agency of Japan in most cases. Samples were captured fresh and identified into species in accordance with Hatooka (2013). A specimen of *Lycodes raridens* from the Bering Sea was kindly donated by A. Yamazaki and H. Munehara, Hokkaido University, Japan. Small muscle tissues were removed from each specimen, immediately placed into 99% ethanol on board and stored in a freezer (-30°C) prior to DNA extraction. Sampling locations and sample data are provided in Table 1-1. The present samples contain 13 *Lycodes* species, five species from the other genera of the subfamily Lycodinae, *Bothrocara*, *Lycenchelys* and *Lycodapus*, three species from the subfamily Zoarcinae and two species from the subfamily Gymnelinae. *Stichaeus nozawai* was used as an outgroup, as the previous phylogenetic study have showed that the family Stichaeidae, to which *S. nozawai* belongs, was an appropriate outgroup for the family Zoarcidae (Miya et al. 2003).

Genomic DNA was extracted using DNeasy Blood & Tissue extraction kit (QUIAGEN) following the manufacturer's protocols. DNA fragments containing mitochondrial cytochrome *b* (cyt*b*), cytochrome *c* oxidase subunit I (COI), 12S rRNA (12S) and 16S rRNA (16S) genes were amplified by PCR, respectively. In addition, DNA fragments containing the nuclear Rhodopsin retrogene (Rhodopsin) were amplified. Primer information is provided in Table 1-2. The PCR consisted of an initial denaturing cycle (94 °C for 2 min), 30 cycles with

denaturation (30 s at 94 °C), annealing (1 min at 47 °C for COI and 50 °C for other genes) and extension (1.5 min at 72 °C), and a final extension step (5 min at 72 °C). PCR products were purified with ExoSAP-IT (United States Biochemical), subsequently sequenced in both directions using BigDye terminator cycle sequencing kit version 3.1 (Applied Biosystems) and ABI 3130xl Genetic Analyzer (Applied Biosystems).

1.2.2. Sequence alignment and characterization of gene fragments

Sequence alignments among species were performed using the program Clustal W implemented in the program package MEGA5 (Tamura et al. 2011) with the default settings. For 16S and 12S, further alignment optimizations were accomplished using the program ProAlign v0.5α (Löytynoja and Milinkovitch 2003) and regions with posterior probabilities lower than 90% were discarded and not used in the following analysis.

To determine the most appropriate nucleotide substitution model for each data partition, the hierarchical likelihood ratio test was performed based on Akaike Information Criterion (AIC; Akaike 1973) using the model test function implemented in MEGA5. I estimated number of characters (*N*), number of parsimony informative site (*S*), nucleotide composition and transition/transversion ration (*K*) under the estimated substitution model using MEGA5 to compare the characteristics among the loci.

Genetic distances based on the 1st, 2nd, and 3rd codon positions were plotted against uncorrected genetic distances based on all codon positions to examine whether the saturation occurred in any substitution sites of protein coding genes. Unsaturated codon positions should show a linear relationship against the total genetic distance. In the case of saturation of substitutions, the relationship shows the plateau in a part with large genetic distances.

1.2.3. Phylogenetic analyses

Phylogenetic relationships among taxa were reconstructed using the maximum-likelihood (ML) and the Bayesian inference (BI) methods. I divided aligned sequences into nine partitions (first, second and third codon positions of cyt*b* and COI; 16S; 12S; Rhodpsin) and subjected each dataset to the partitioned maximum-likelihood analysis using the program RAxML v7.26 (Stamatakis 2006). A general time reversible model with sites following a discrete gamma distribution (GTR + Γ; the model recommended by the author) was used on a dataset with nine partitions and a rapid bootstrap analysis was conducted with 1,000 iterations. BI was performed using the program MrBayes v3.2 (Ronquist and Huelsenbeck 2003). Runs were recorded every 100 steps out of a total of 10,000,000 steps of Markov Chain Monte Carlo (MCMC), with a burn-in of 2,500,000 steps (i.e., 25%). Metropolis Coupling using 4 chains was employed with heating increment of 0.2 to ensure that analyses were not trapped on local optima. The results were checked using the program TRACER v1.5 (Rambaut and Drummond 2009) by testing that effective sample sizes for all demographic statistics were above 200 for each run. All trees were rooted using the outgroup (*S. nozawai*) and tree topologies were compared. I also reconstructed the phylogenetic relationships among the species in the subfamily Lycodinae based on mitochondrial cyt*b* and 12S, using the additional nucleotide sequences, which were previously reported by Møller and Gravlund (2003) to maximize the explanatory power of the evidences. The sequence data of 18 additional *Lycodes* species were downloaded from GenBank (for Accession numbers, see Table 1-3), combined with 24 taxa obtained in this study and analyzed in the same procedures described above.

I performed a parsimony-based reconstruction of evolutionary process of a submental crest based on a strict consensus of the ML and BI trees, using the program Mesquite v2.6 (Maddison and Maddison 2009). Two character states of the submental crest, namely, “present” (state 1) and “absent” (state 2) were assigned to each species, based on present systematics of the family Zoarcidae (Anderson 1994).

1.2.4. Divergence time estimation

In order to better understand the evolutionary history of the subfamily Lycodinae, the clock calibrated tree was required. The program BEAST v1.8.0 (Drummond and Rambaut 2007) was used to reconstruct the clock calibrated tree. I performed MCMC analyses which consist of 100,000,000 generations with sampling each 1,000 generations and first 10,000,000 generations (i.e., 10%) were discarded as a burn-in. The sequence data from five genes were used with independent evolutionary models for each gene and with empirical base frequencies. In the case that the best-fit evolutionary model was not implemented in the BEAST (e.g., Tamura's three parameter model and Kimura's two parameter model), the second- or third-best models were used. Yule process speciation model was used as the tree prior. Fossil record of zoarcid species have not been reported and thus the age of divergence of zoarcid taxa cannot be estimated based on paleontological data. Although any molecular evolutionary rates has not been reported for zoarcid fishes, the rate of cyt*b* of a gasterosteid fish (the three-spined stickleback, *Gasterosteus aculeatus*) which is a related group of Zoarcidae (Miya et al. 2003; Near et al. 2012), was reported to be 2.045% per My (Mäkinen and Merilä 2008). Thus we modeled the substitution rate of the cyt*b* under the strict clock model, with a normal distribution with mean and standard deviation of 2% and 1% per site and My, respectively. For the rate evolution model for other loci, the uncorrelated log-normal relaxed clock model was selected. The results were checked using the program TRACER v1.5 (Rambaut and Drummond 2009) by testing that effective sample sizes for all statistics were above 200.

1.3. Results and discussion

I describe and discuss the phylogenetic relationships and evolutionary history of the species of Lycodinae in the following section. This study represents robust and comprehensive phylogenetic relationships among deep-sea fishes of the subfamily Lycodinae, using four

mitochondrial and one nuclear loci.

1.3.1. Characteristics of the five genes

I determined partial sequences of four mitochondrial and one nuclear genes from 24 taxa. All sequences determined in this study were presented in Table S1 in the Appendix. The two protein coding genes (cyt*b* and COI) did not show any evidence of saturation (Fig. 1-1). Among five genes examined in this study, cyt*b* was most informative and Rhodopsin was most conserved (Table 1-4). There seems to be anti-G bias in cyt*b* and COI, which was also reported for other teleost fishes (Hyde and Vetter 2007; Kai et al. 2003). Rhodopsin was characterized by a relatively low transition/transversion ratio, probably because the nuclear non-coding retrogenes are relatively free from the functional constraints of coding regions (Armstrong et al. 2001; Birks and Edwards 2002).

Although various substitution models were suggested by MEGA5, only 3 substitution models were implemented in MrBayes (GTR, HKY and Jukes-Cantor) and GTR model was recommended with higher AIC than Jukes-Cantor and HKY for all partitions in MEGA5. Therefore, the GTR substitution model with a gamma-distributed rate variation across sites and a proportion of invariable sites (GTR + Γ + I) was used on a dataset with nine partitions in the BI.

1.3.2. The relationships between subfamilies of the family Zoarcidae

The phylogenetic relationships among 23 zoarcid species strongly support monophyly of two clade formed by species of the subfamilies Lycodinae and Zoarcinae, respectively (Fig. 1-2: for independent ML and BI trees, see Figs. 1-3 and 1-4). Phylogenetic relationships among 41 species including 18 additional species are also shown in Fig 1-5. The monophyletic clade of *D.*

poecillimon and *K. maculata* was also weekly supported by both analyses. Among four subfamilies in the family Zoarcidae, which Anderson (1994) established on the basis of the cladogram using morphological characters, a remaining subfamily Lycozoarcinae could not be included in this study as a sole species of this subfamily *Lycozoarces hubbsi* was not available. Therefore, I could not determine the complete relationships among subfamilies.

1.3.3. Systematics of the subfamily Lycodinae

Present results clearly show that the genus *Lycodes* is a polyphyletic group (Fig. 1-4). Within the, I subfamily Licodinae recognized three clades containing species of *Lycodes*: Clade A including four *Lycodes* and a single *Lycenchelys* species; Clade B including three *Lycodes* and two *Lycenchelys* species; Clade C including five *Lycodes* species, which formed a clear monophyletic group, and two species with gelatinous body tissues, namely, *Bothrocara hollandi* and *Lycodapus microchir* (Fig. 1-5). The monophyly of the genus *Lycodes* has been generally accepted primary based on morphological analyses. Previous molecular phylogenetic analyses also supported this conclusion (Møller and Gravlund 2003). The molecular analyses by Møller and Gravlund (2003), however, did not use samples from the Far East, which is included in the Clades A and B in Fig. 1-4. The phylogenetic reconstruction using the previously reported sequence data of 12S and cyt*b* genes strongly suggests that their taxon sampling was insufficient (Fig. 1-5).

Lycenchelys, the second largest genus in the subfamily Lycodinae, was also inferred to be polyphyletic in the present study (Fig. 1-4). Anderson (1994) classified 57 species into *Lycenchelys*, which are distributed worldwide, based on the combination of several morphological characters. He also stated that the morphological differences between *Lycodes* and *Lycenchelys* were slight. Among three *Lycenchelys* species used in this study, *L. melanostomias* and *L. albomaculatus* were first described by Toyoshima (1983) and *L. aurantiaca* was classified into *Lycenchelys* by Shinohara (1998) based on the resemblance in

general appearance to *L. alta* and *L. squamosus*, which had been also described by Toyoshima (1985). Although the present data do not allow me further discussion on the taxonomy of the genus *Lycenchelys*, I suggest that the comprehensive review is required for the systematics of this genus.

Toyoshima (1985) recognized the genus *Petroschmidtia* represented by two species *P. toyamensis* and *P. albonotata*, which was distinguished from the other zoarcid species by the combination of three morphological characters: 1) no pyloric caeca; 2) no vomerine or palatine teeth; and 3) the unique lateral pelvic "spine" (Matsubara and Iwai 1951). Anderson (1994) stated more recently that only the last character was unique to these species and concluded that the genus *Petroschmidtia* was invalid. These two species, which Anderson (1994) had included in the genus *Lycodes*, however, formed a monophyletic clade within the Clade A in Fig. 1-4. Shinohara (pers. comm.) noticed lateral pelvic spines for *L. teraoi* and *L. sadoensis*. These two species also formed a monophyletic group with *L. toyamensis* and *L. albonotata* while a lateral pelvic spine have not been reported for *Lycenchelys melanostomias*, the last member of the Clade A. I therefore suggest that the genus *Petroschmidtia* is a valid taxon containing at least four species of *P. toyamensis*, *P. albonotata*, *P. teraoi* and *P. sadoensis*.

Several species of *Lycodes* exhibit a notch in the pectoral fin, and it has long been used as a character for grouping species (e.g., the subgenus *Furcimanus*, Jordan and Evermann 1898). Stevenson and Sheliko (2009) treated *L. nakmurai*, *L. pectoralis*, *L. diapterus*, *L. nishimurai* and *L. hubbsi* as members of the subgenus *Furcimanus*. Later, Anderson (1994) suggested that *L. hubbsi* may be a junior synonym of *L. diapterus*. It is, however, evident that the subgenus *Furcimanus* is polyphyletic (Figs. 1-2 and 1-3). Stevenson and Sheliko (2009) also suggested that *L. eudipleurostictus* was closely related to *L. hubbsi* because two species showed similar meristics and color patterns. The fact that the two species are distinct from each other (Fig. 1-5) rejects this assumption, and infers that notches in the pectoral fin of *Lycodes* species might be homoplastic. Among above mentioned *Furcimanus* species, a close relationship between *L. nakamurai* and *L. pectoralis* was recognized in the present results. *L. pectoralis*, which was first described by Toyoshima (1985) as a closely related species of *L. diapterus*, and

the relationships between *L. pectoralis* and *L. nakamurai* was not discussed. Stevenson and Sheliko (2009) later suggested that *L. pectoralis* resembled *L. nakamurai* rather than *L. diapterus*. The present data showed that sequence divergence between *L. nakamurai* and *L. pectoralis* was 0.6% in COI (See Table S1 in the Appendix), which suggests that the validity of two species is questionable, as the range of COI sequence divergence between sibling species of the Chordata was previously reported to be within the range of 1.2–12.9% (Hebert et al. 2003).

The parsimony-based reconstruction of the ancestral character state showed that the submental crests, which characterize the genus *Lycodes*, occurred in three lineages, independently (Fig. 1-6). Although the function of the submental crest was not clarified, Taranetz and Andriashev (1934) described the well-developed submental crest of *L. albonotata* as a specialized feature that is adaptive to inhabit the muddy deep-sea bottom. The species of the genus *Lycodes* are widely distributed on the muddy bottoms of continental shelves and slopes (Anderson 1994), and the submental crests might have contributed to the present diversity of these species.

1.3.3 Biogeography of the subfamily Lycodinae

In the present results, I found several pairs of sibling species, which infers that the past geological history had strongly influenced the diversification process of species of the subfamily Lycodinae. *L. japonicus* and *L. ocellatus* were suggested to be a pair of sibling species that show parapatric distribution in the Clade B (Figs. 1-2 and 1-3). Four species in the Clade B were reported from the northwestern Pacific off the northern Japan, except *L. japnicus*, which is endemic to the Sea of Japan. Based on the principle of parsimony, *L. japnoicus* likely invaded the Sea of Japan from the ancestral population in the northwestern Pacific Ocean and subsequently speciation occurred. It was impossible to determine the ancestral distribution of the species in the Clade A, however, it seemed that the migration between the Sea of Japan and the Sea of Okhotsk have occurred at least twice. Assuming sibling relationships among the

species of the Clade C was also difficult because all *Lycodes* species which were included in the previous molecular phylogenetic study belong to Clade C and I could not determine the accurate interspecific relationships (Fig. 1-6).

The molecular-clock Bayesian analysis of the divergence time provided the additional information about the evolutionary history of the subfamily Lycodinae (Fig. 1-7). The pairs of sibling species were suggested to have diverged during the last one My, when the glacial climate changes were drastic. The northwestern Pacific is characterized by many marginal seas, which have been affected by environmental changes through the Pleistocene glacial cycle. Drastic sea-level changes have repeatedly isolated and reconnected the deep-sea habitats in the marginal seas during the last one My. It was also suggested that the three lineages of *Lycodes* species may have synchronously diverged from "non-*Lycodes*" ancestral lineages of the subfamily Lycodinae during the early Pleistocene (Fig. 1-7).

It has been suggested that some groups of deep-sea demersal fishes inhabiting northwestern Pacific marginal seas have diversified by allopatric speciation under the strong influences of the glacial climatic oscillations (Nishimura 1968; Hyde and Vetter 2007). Divergence time estimates in this study was not based on the fossil records, which possibly makes the conclusions of this study very weak. However, these results are concordant with the previous studies which inferred the mid- to late- Pleistocene divergence of the *Lycodes* species in the Clade C (Fig. 1-7) and I assume that the present results might be reasonable.

My findings infer that allopatric speciation have contributed to the present diversity of the subfamily Lycodinae. I will further discuss the influences of the Pleistocene climate changes on the allopatric divergence of the species in the subfamily Lycodinae in the Chapter 3.

Tables

Table 1-1 List of specimens used in phylogenetic reconstruction, sampling locations and collection numbers.

Species name		Collection location				
		Latitude (N)	Longitude (E)	Depth (m)	Date	Collection number
Bothrocara	*hollandi*	Niigata Pref., Japan			Feb 2012	
Davidijordania	*poecillimon*	35˚02 N	135˚05 E	182	Apr 2011	
Krusensterniella	*maculata*	Shimane Pref., Japan			NA	FAKU131970[*1]
Lycenchelys	*albomaculata*	44˚16 N	144˚58 E	764	May 2011	
Lycenchelys	*aurantiaca*	39˚02 N	142˚14 E	650	Oct 2011	
Lycenchelys	*melanostomias*	44˚14 N	144˚35 E	834	May 2011	
Lycodapus	*microchir*	37˚38 N	142˚01 E	510	Nov 2009	
Lycodes	*albonotata*	44˚51 N	143˚55 E	472	Apr 2012	
Lycodes	*hubbsi*	Fukushima Pref., Japan			Oct 2009	
Lycodes	*japonicus*	Niigata Pref., Japan		NA	Feb 2012	NSMT-P111910[*2]
Lycodes	*matsubarai*	43˚31 N	143˚01 E	212	Apr 2012	
Lycodes	*nakamurai*	36˚16 N	132˚38 E	440	May 2012	
Lycodes	*ocellatus*	38˚22 N	142˚ 14 E	750	Oct 2009	NSMT-P111913[*2]
Lycodes	*pectoralis*	43˚02 N	143˚ 55E	207	Apr 2012	
Lycodes	*raridens*	70˚36 N	161˚26 E		Jul 2013	
Lycodes	*sadoensis*	NA			Apr 2013	
Lycodes	*soldatovi*	44˚51 N	143˚55 E	472	Apr 2012	
Lycodes	*tanakae*	38˚01 N	136˚55 E	330	Jun 2011	
Lycodes	*teraoi*	36˚25 N	133˚38 E	220	May 2012	
Lycodes	*toyamensis*	36˚45 N	136˚06 E	373	Jun 2010	
Stichaeus	*nozawai*	44˚05 N	144˚31 E	234	May 2011	
Zoarces	*americanus*	New England, USA			2008	NSMT-P102511[*2]
Zoarces	*elongatus*	Hokkaido, Japan			Jul 2010	NSMT-P102227[*2]
Zoarces	*gilli*	NA			NA	FAKU134658[*1]

*1 The fish collection of Kyoto University (FAKU)

*2 National Museum of Nature and Science, Tokyo (NSMT)

Table 1-2 PCR primers used in this study for DNA amplification.

Gene	Name	Sequence (5'–3')	Reference
cyt*b*	Glu-DGL	TGA CTT GAA RAA CCA YCG TTG	Palumbi 1996
	CB3H	GGC AAA TAG GAA RTA TCA TTC	Palumbi 1996
COI	LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. 1994
	HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. 1994
12S	12SA	AAA CTG GGA TTA GAT ACC CCA CTAT	Kocher et al. 1989
	12SB	GAG GGT GAC GGG CGG TGT GT	Kocher et al. 1989
16S	16SarL	CGC CTG TTT ATC AAA AAC A	Palumbi et al. 1991
	16SbrH	CGG GTC TGA ACT CAG ATC ACG	Palumbi et al. 1991
Rhodopsin	Rh193f	CNT ATG AAT AYC CTC AGT ACT ACC	Chen et al. 2003
	Rh1039r	TGC TTG TTC ATG CAG ATG TAG A	Chen et al. 2003

Table 1-3 GenBank accession numbers of the nucleotide sequences of cytochrome *b* (cyt*b*) and 12S rRNA (12S) previously deposited by Møller and Gravlund (2003). These sequences were used in the phylogenetic reconstruction in this study.

Species name		GenBank Accession No.	
		12SrRNA	Cytochrome *b*
Lycodes	*adolfi*	AY159965	AY158832
	concolor	AY159959	AY158830
	cortezianus	AY159956	AY158827
	diapterus beringi	AY159957	AY158828
	diapterus diapterus	AY159958	AY158829
	esmarkii	AY159972	AY158833
	eudipleurostictus	AY159967	AY158835
	luetkenii	AY159970	AY158824
	mcallisteri	AY159971	AY158823
	paamuiti	AY159968	AY158822
	pacificus	AY159960	AY158821
	palearis	AY159955	AY158826
	pallidus	AY159962	AY158820
	reticulatus	AY159966	AY15881
	rossii	AY159975	AY158818
	seminudus	AY159973	AY158817
	terranovae	AY159969	AY158816
	vahlii	AY159974	AY158834

Table 1-4 Characteristics of the cytochrome *b* (cyt*b*), cytochrome *c* oxidase subunit I (COI), 12S rRNA (12S), 16S rRNA (16S) genes and nuclear Rhodopsin retrogene (Rhodopsin). The final length of sequences used in the analyses (*N*), the number of parsimony informative sites and its composition in each locus (*S*), best-fit nucleotide substitution model suggested by MEGA5, nucleotide base composition and transition/transversion ratio (*K*) are presented.

Gene	*N*	*S*	Model	Nucleotide composition				*K*
				%A	%T	%G	%C	
cyt*b*	714	194 (27%)	TN93+Γ+I	21.7	29.6	17.1	31.6	5.94
COI	627	146 (23%)	K2P+Γ+I	21.1	27.9	19.9	31.1	6.46
12S	360	39 (11%)	K2P+Γ	27.3	20.5	25.2	27.1	4.05
16S	322	51 (16%)	K2P+I	28.7	23.7	24.3	23.4	4.46
Rhodopsin	759	41 (5%)	T92+Γ+I	16.2	23.8	27.4	32.6	0.88

Figures

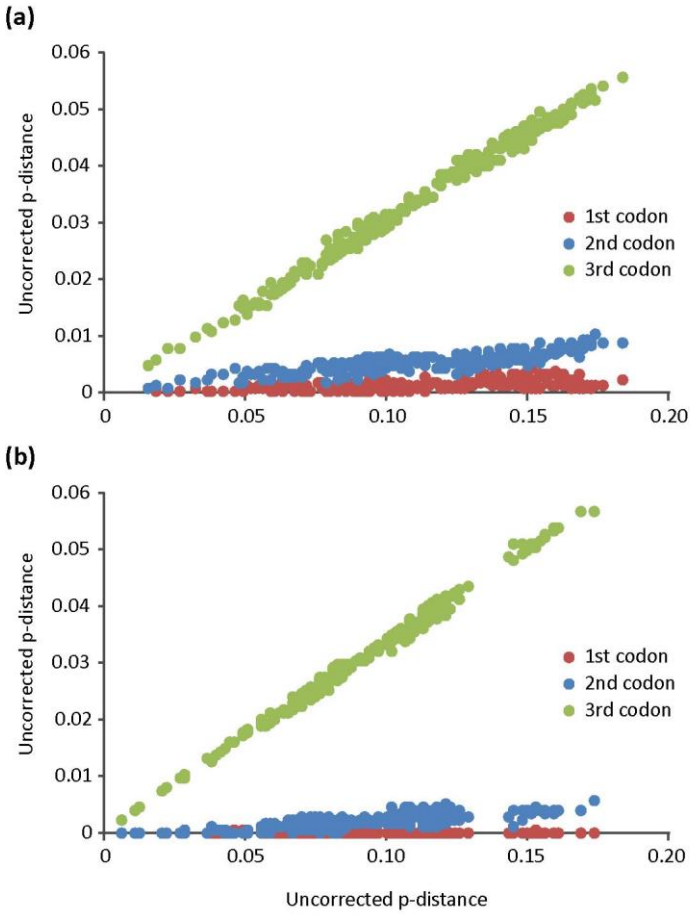

Fig. 1-1 Graphical presentation of the pairwise distance at 1st (red), 2nd (blue), and 3rd (green) codon positions as a function of uncorrected p-distance for the cytochrome *b* (cyt*b*: a) and cytochrome *c* oxidase subunit I (COI: b) genes.

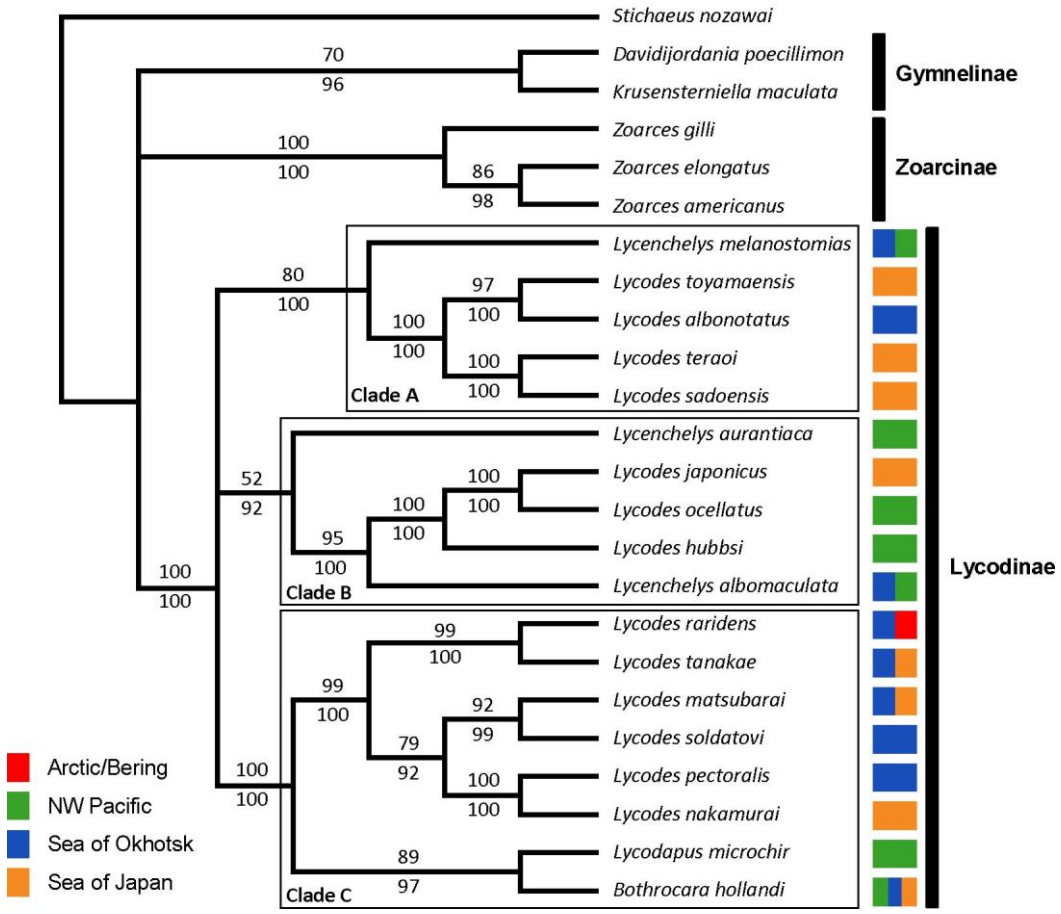

Fig. 1-2 A strict consensus of the maximum likelihood (ML) and Bayesian inference (BI) trees, indicating the geographic distribution of Licodinae species. Nodal support was shown as percentages by bootstrap probabilities based on 10,000 iterations of ML (>50%, above branches) and the posterior probabilities of BI (>80%, below branches). The three major clades among the family Lycodinae were also shown (Clades A to C).

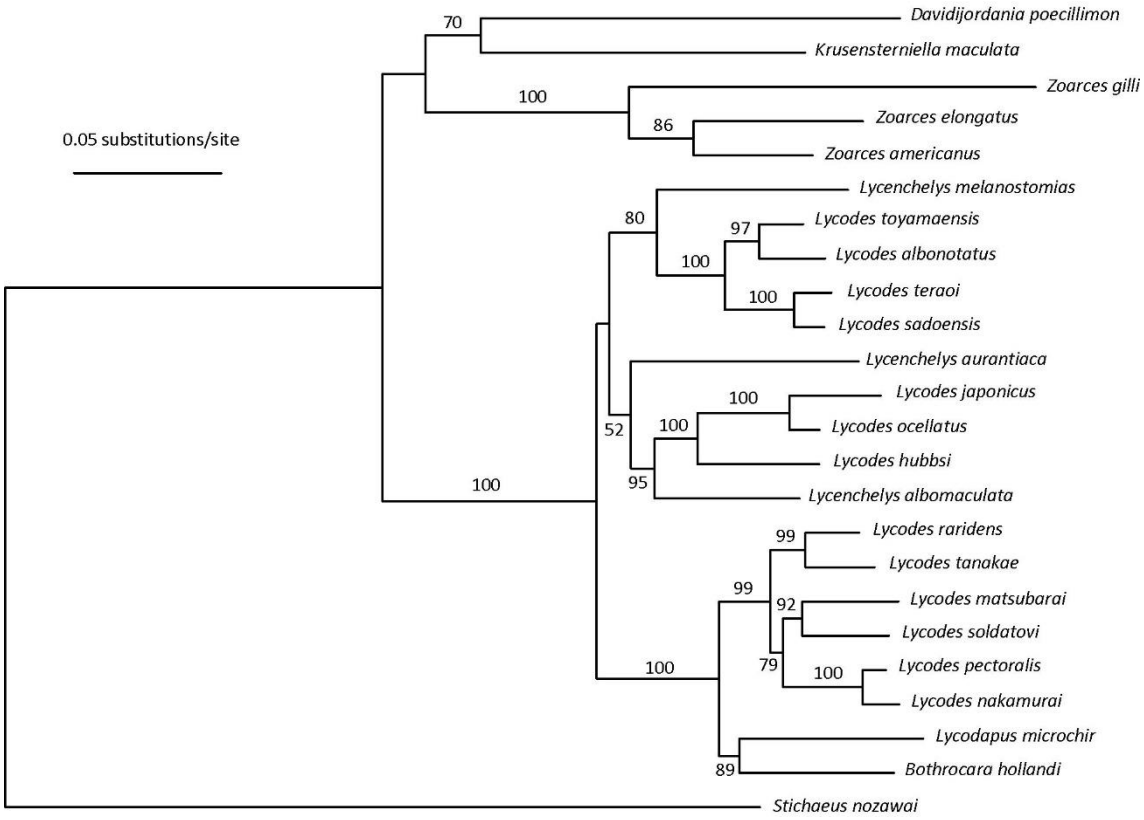

Fig. 1-3 The best-scoring maximum likelihood (ML) tree based on 10,000 iterations. Numerals beside branches indicate bootstrap probabilities of ML > 50%.

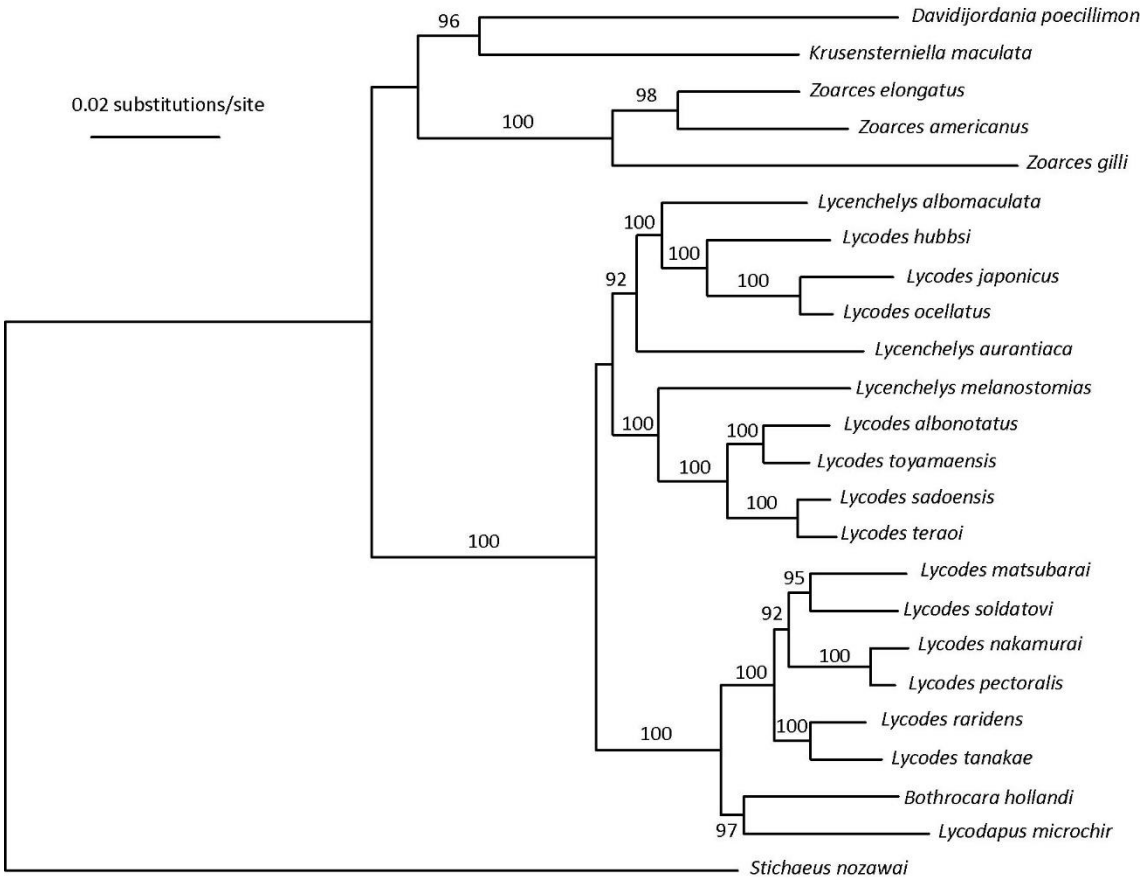

Fig. 1-4 A consensus tree with maximum posterior probability from Bayesian posterior analysis (BI). Numerals beside branches indicate Bayesian posterior probabilities > 80%.

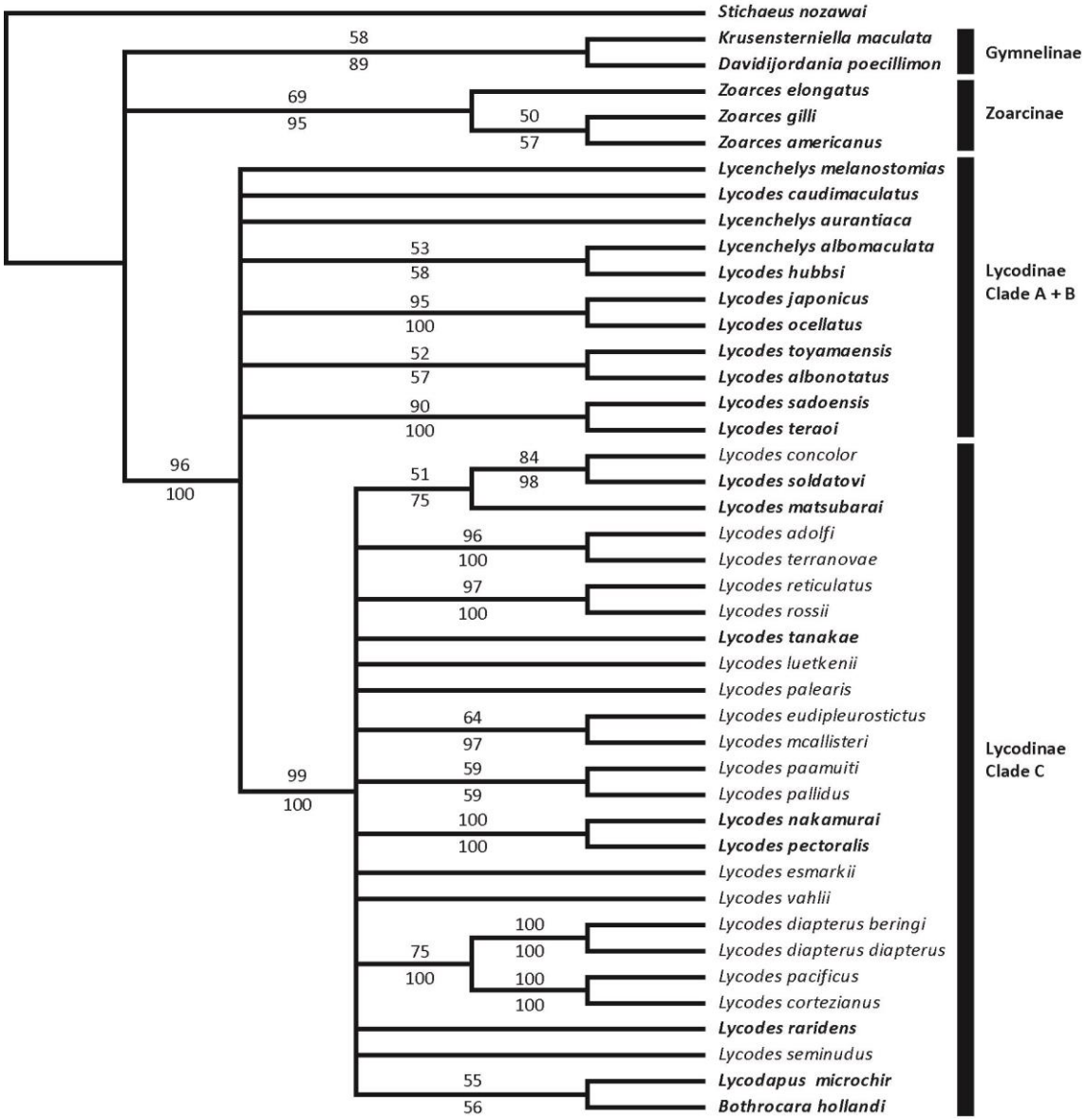

Fig. 1-5 A strict consensus of the maximum likelihood (ML) and the Bayesian inference (BI) trees using the sequence data from cytochrome *b* (cyt*b*) and 12S rRNA (12S) genes. In addition to the data obtained in this study, the data from Møller and Gravlund (2003) was supplemented. Nodal support was indicated as percentages by bootstrap probabilities based on 10,000 iterations in ML (above branches) and the posterior probabilities in BI (below branches). Bold characters indicate the species whose sequence data were obtained in this study.

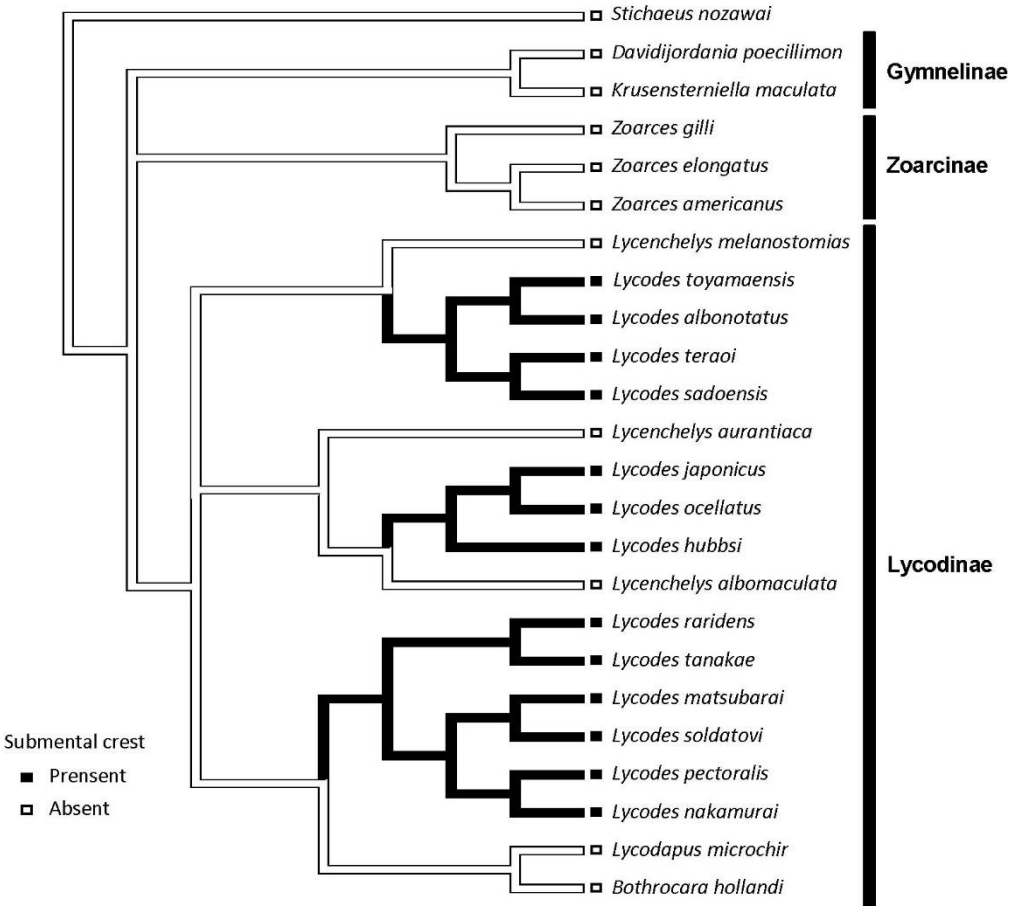

Fig. 1-6 Parsimony based reconstruction of evolutionary process of submental crests in zoarcid fishes. Two discrete character states (absent or present) were assigned to each terminal and ancestral character states were reconstructed on the ML tree (Fig. 1-2) using Mesquite v2.6 (Maddison and Maddison 2009).

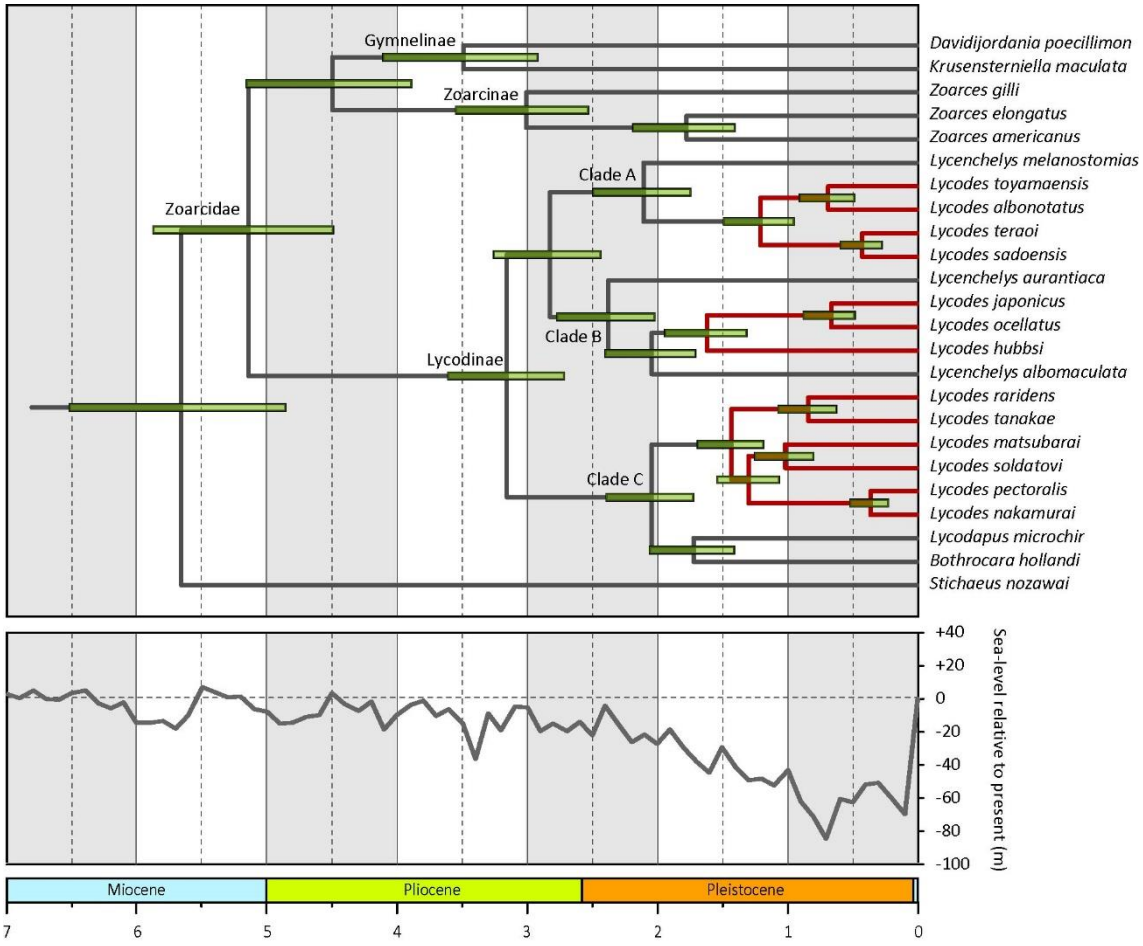

Fig. 1-7 Divergence times of the lineages of Zoarcidae, estimated using BEAST v1.8.0. Horizontal bars indicate 95% posterior density intervals of the divergence time estimation. The clades indicated by red lines represent the lineages of *Lycodes* species. Global trends of sea-level change during the last seven million years (My), averaged over 100,000 year intervals, were also shown (modified from Miller et al. 2005). Dashed line indicates the present sea-level.

Chapter 2

Distribution and species composition of deep-sea fishes of the subfamily Lycodinae in the southwestern Sea of Japan

2.1. Introduction

The Sea of Japan is one of the marginal seas of the northern Pacific Ocean. This sea is semi-enclosed and its deep-sea part is isolated from the neighboring Pacific Ocean, the Sea of Okhotsk and the East China Sea. The deep-sea ichthyofauna of the Sea of Japan is characterized by low species diversity and the rarity of endemic species, which are attributed to the severe anoxic conditions in most of the deep-sea parts of this sea area during the LGM (Nishimura 1968; Tyler 2002). Only 20 fish species were recognized from the deep-waters below 300 m (Zenkevitch 1963; Okiyama 2004). In the southern areas of the Sea of Japan, the Tsushima Current bringing warm water through the Tsushima Strait and the warm water is mixed with the cold deep water mass called the Japan Sea Proper Water on the continental slopes (Sudo 1986). The patterns of faunal zonation in the continental shelves and slopes in the Sea of Japan might be strongly influenced by the hydrographic structure, however, the spatial patterns of faunal zonation in relation to such environmental conditions were poorly known.

The genus *Lycodes* (Perciformes: Zoarcidae) is a large group of a deep-sea demersal fishes encompassing approximately 60 species. Most of *Lycodes* species inhabit the deep-sea floor on the continental shelves and slopes in the northern Pacific, Arctic and northern Atlantic Oceans (Anderson 1994). The southwestern Sea of Japan is one of sea areas where *Lycodes* species are overwhelmingly dominant. Among the poor ichthyofauna, six species of *Lycodes* were known, namely, *L. tanakae*, *L. matsubarai*, *L. nakamurai*, *L. toyamensis*, *L. teraoi* and *L. japonicus*. Some of them were reported to occur sympatrically (Okiyama 2004), while the geographic range of each species needs to be closely examined.

In this study, the detailed geographic and bathymetric distribution and the size frequency distributions of the six *Lycodes* species in the southwestern Sea of Japan were investigated. To estimate which environmental factors contribute to the geographic change of the species composition, the relationships between environmental variables and the composition

of *Lycodes* species were also examined under the Distance-based liner model (DistLM: Anderson et al. 2008). Present results show the local changes in a regional assemblage of the deep-sea fishes within the Sea of Japan and effects of some environmental factors on the spatial community structure of the deep-sea demersal fishes.

2.2 Materials and methods

2.2.1. Sampling procedures

The sampling was conducted in March, June and July 2012 during the cruise of the training ship (T/S) Tanshu-Maru (Kasumi High School, Hyogo Prefecture) when the fisheries surveys were carried out by the Japan Sea National Fisheries Research Institute, Fisheries Research Agency. *Lycodes* species were collected using an otter trawl towed from double warps at 141 stations along the upper continental slope (179–497 m) in the southern Sea of Japan. The net body was constructed of 50 mm stretched mesh polyethylene net and the cod-end was lined with 15 mm stretched-mesh liner. Each tow had a standard duration of 30 min, but in some case they were shortened. A net mensuration equipment was used for measuring net spread (mean width: $\overline{w} =$ 17.74 m) and net height (mean height: $\overline{h} =$ 6.35 m). The global positioning system (GPS) was used to determine the towed distance. The trawling areas were calculated based on the values of the net spreads and the towed lengths. All *Lycodes* specimens were identified to the species level in accordance with Hatooka (2013), and then counted. A density of *Lycodes* species was expressed as the number of fish per a square kilometer swept by the trawl (no./km^2). Total lengths (TL) of all specimens were also measured on board at all stations to the nearest 1 mm. The salinity and temperature of bottom layer was recorded with a compact salinity, temperature and depth sensor (STD: JFE Advantec Co., Ltd.) at each sampling station.

2.2.2. Statistical analyses

For statistical tests, sampling stations were grouped into five regions (A–E in Fig. 2-1). Kafanov et al. (2000) investigated the ichthyofauna in the Sea of Japan and reported that a faunistic boundary between the warm-temperate and mild-temperate zones established between 36–37° N, which corresponds to the long-term average of annual minimum surface temperature of 10.5°C. Although data from the deep-sea fauna below 200 m was used, they did not discuss the faunistic boundary in the deep-water (Kafanov et al. 2000). Thus, to test whether the faunistic boundary proposed by Kafanov et al. (2000) is valid for the deep-sea area, we divided the sampling stations into two groups on both sides (north and south) of the latitude line of 36.5° N (A, B and D vs. C and E, Fig. 2-1). Furthermore, stations in these two groups were subdivided into 2 and 3 subgroups (regions), respectively, corresponding to grids (regions) spacing of 2° of longitude as shown in Fig. 2-1. Each region was further divided into the components within four depth ranges (150–190m, 190–300m, 300–400m and 400–500m; Table 2-1). The average values of environmental variables (water depth, salinity and temperature) among stations of each of the resultant 16 components (Table 2-1) were used for statistical tests. Two components A150 and B150, where no specimens were collected, were not included in the analysis.

To visualize resemblances in the composition of *Lycodes* fishes among the components, non-metric multi-dimensional scaling (nMDS) was conducted (Clarke and Warwick 2001). Prior to analyses, density of each *Lycodes* species was log (X + 1)-transformed and the Brey-Curtis index was used as a similarity index. In addition, we evaluated how well the environmental variables explain the spatial variation of species composition by the distance based linear models (DistLM: Anderson et al. 2008). The forward selection of the predictor variables were carried out with 9,999 permutations of marginal tests based on Akaike Information Criterion (AIC: Akaike 1973). Removal of one of two variables with high correlation coefficient ($|r| > 0.95$) is recommended in the DistLM analysis (Anderson et al. 2008). Because the highest correlation, which was detected between water temperature and salinity, was 0.90, all environmental variables, namely, water depth, salinity and temperature, were included in this analysis. These multivariate analyses were performed using PRIMER6 with PARMANOVA+, an add-on package (PRIMER-E Ltd., Plymouth, UK).

2.3. Results

During the all trawl surveys in 2012, the temperature and the salinity changed drastically between the depths of 200 and 250 m (Table 2-1 and Fig. 2-2). The average temperature varied from 1.2 to 6.1°C within the areas shallower than 300 m (Table 2-1). In the area deeper than 300 m, the salinity (34.0–34.1‰) and the temperature (0.3–1.0°C) were relatively constant, probably due to the exceedingly cold Japan Sea Proper Water.

A total of 7,518 specimens (1,182 kg, wet weight) of *Lycodes* fishes were obtained from 116 stations in the southwestern Sea of Japan (Table 2-2). The mean density of each *Lycodes* species at each component is shown in Table 2-3. No *Lycodes* fish was collected at the two western shallow components (A150 and B150).

2.3.1. Geographic distribution

The six *Lycodes* species showed species-specific patterns in geographic distribution (Fig. 2-3). *L. tanakae* was the most common and was obtained from almost all components (Fig. 2-3a and Table 2-3). *L. matsubarai* showed a patchy distribution (Fig. 2-3b) and was dominant in the westernmost grid A (Table 2-3). *L. toyamensis* and *L. nakamurai* were more abundant than the other species (Table 2-2). They were obtained from a broad range in the study area, showing similar geographic distribution; these two species were not collected in the grid A (Fig. 2-3c, d and Table 2-3). Among six species, density of two dwarf species *L. japonicus* and *L. teraoi* (Toyoshima 1985) was relatively low and a total weight of each of them (1 kg) was also the smallest (Fig. 2-5e and f). The geographic range of *L. japonicus* was almost limited to the grid D and *L. teraoi* was obtained mainly from the grids B, C and D (Fig. 2-3e, f and Table 2-3).

2.3.2. Bathymetric distribution

Six *Lycodes* species showed different modes of bathymetric distribution (Fig. 2-4). *L. tanakae*

was abundant in the depth range of 250–300 m and its density decreased as the water depth increased (Fig. 2-4a). *L. matsubarai* was mainly distributed in the depth range of 300–450 m but some specimens were obtained from the range of 200–250 m (Fig. 2-4b). *L. toyamensis* and *L. nakamurai* showed a slightly different bathymetric distribution: *L. toyamensis* showed the peak of density in the depth range of 350–400 m while the peak of *L. nakamurai* was in the range of 300–350 m (Fig. 2-4c, d). *L. japonica* was distributed in the range of 300–500 m and *L. teraoi* was obtained only from the water shallower than 300 m (Fig. 2-4e, f).

2.3.3. Size composition

The size frequency distributions of six *Lycodes* species were shown in Fig. 2-5. *L. tanakae* was the largest (mean TL = 407 mm). Although the mode of the TL appeared at 270–280 mm for both *L. nakamurai* and *L. toyamensis*, the mean value of the latter was significantly larger than *L. nakamurai* (t-test, $p<0.01$). For each species, the length frequency distribution did not show any signatures of the cohorts.

The relationships between the depth of each sampling stations and the TL of the smallest individuals obtained from the sampling station are shown for each species in Fig. 2-6. *L. tanakae* and *L. toyamensis* showed significant negative and positive correlation between the depth and the size, respectively ($p<0.01$). For the other four species, no significant relationship between them was detected.

2.3.4. Resemblance among local assemblages

The results of the nMDS analysis showed that the 16 components were grouped into four local assemblages under the similarity of 70% (Fig. 2-7). Two components in the westernmost grid A were clearly distinguished from all other components and characterized by the dominance of *L. matsubarai*. The component B190, a shallow component of the grid B was also distinct from others, since the most specimens of *L. teraoi* were collected in this component. The component

E190 was also segregated from the others. Remaining components formed a single group under the similarity of 70%. Although three subgroups were recognized under the similarity of 80% within this group, neither clear geographical nor bathymetric character was recognized for each subgroup (Fig. 2-2).

2.3.5. Species composition and environmental factors

Results of the DistLM carried out using the Bray-Curtis similarity matrix was shown in Table 2-4. The DistLM selected all three variables as predictor of the differences in species composition and the two variables, namely, bottom water temperature and salinity significantly explained approximately 50 % of the variation.

2.3. DISCUSSION

2.4.1. The distribution of each Lycodes *species*

Present results reveal the geographic and bathymetric distribution, and size composition of *Lycodes* species in the southwestern Sea of Japan in detail. Each of *Lycodes* species showed a species-specific pattern in the geographic distribution. Among the six species, the two dwarf species (*L. japonicus* and *L. teraoi*) showed restricted distribution ranges, while *L. tanakae*, which was the largest in six species, was distributed in the most part of the study area. Positive relationships between body size and range size were also reported for freshwater fishes (Taylor and Gotelli 1994; Pyron 2001) and the various mechanisms were proposed to explain the relationships (Gaston and Blackburn 1996).

The significant relationships were detected between the depth of the sampling stations and the TL of the smallest individuals collected at each sampling station for *L. tanakae* and *L. toyamensis*, which may infer the vertical migration of the two species. Adults of *L. tanakae* were suggested to migrate from the deep wintering places toward the shallow continental

shelves in the summer and return to the lower part of continental shelves in the winter (Balanov and Solomatov 2008; Saveliev et al. 2011). In the present study, the vertical migration of adults toward the shallower waters was also suggested for *L. toyamensis*. The result indicates that the reproduction of *L. toyamensis* takes place in the deeper areas. In addition, small individuals of *L. toyamensis* were not obtained in the present surveys (Fig. 2-5). Okiyama (2004) reported *L. toyamensis* from the depth of 1,400 m in the Sea of Japan with referring to the localization of smaller individuals of this species in deeper area. Further survey in the lower part of the continental shelves is desired to reveal the entire lifecycle of this species.

2.4.2. The spatial structure of Lycodes *assemblages in the southwestern Sea of Japan*

In the present study, the local variations of composition within the assemblage of *Lycodes* species in the southwestern Sea of Japan were shown. The differences in the species composition between components were more conspicuous in the shallow than in deep waters (Fig. 2-7). The Japan Sea Proper Water, which occupies the deep layer between the depth of 300 m and deeper than 3000 m, is well mixed by the slow eddy turbulence (Tyller 2002). Such a spatial pattern possibly reflects the uniformity of the environmental condition in the deep-sea areas (Fig. 2-2), which might prevented the formation of spatial structure within the deep layer.

The spatial structure of the *Lycodes* assemblage in the southwestern Sea of Japan was shown to be affected by the environmental factors, namely, water temperature and salinity (Table 2-4). *Lycodes* is a group of cold-water fishes (Anderson 1994) and therefore, the warm Tsushima Current flowing along the continental shelves may strongly restrict the distribution of these species. The fact that no *Lycodes* specimen was obtained from the westernmost stations, probably under the strong influences of the Tsushima Current, supports this assumption (Fig. 2-3). The variation in surface temperature affects surface primary production and thus the supply of foods to the deep seabed (Nishimura 1983), which may also influences the deep-sea fauna.

The faunistic boundary proposed by Kafanov et al. (2000) may not be valid for the deep-sea area in the southwestern Sea of Japan. Among four groups recognized in the nMDS

analysis, the group represented by the component E190 likely corresponds to the mid-temperate zones in Kafanov et al. (2000). However, the shallow component in another region corresponds to the mild-temperate zone, the component C190 was not grouped with E190. The species composition of local assemblages gradually changed rather in the longitudinal than in the latitudinal direction in the shallow waters. In addition, we could not find any evidence of the bioclimatic zoning in the deep-sea areas. Therefore, the gradual changes of water temperature along the warm Tsushima Current possibly contribute to the gradual faunal changes in shallow waters, but this may not the case for deep waters.

The six *Lycodes* species showed species-specific spatial distribution. The assemblage of the deep-sea *Lycodes* fishes was localized in the shallow area, where the Tsushima Current strongly influences. Further studies on the deep-sea megafaunal assemblages in the Sea of Japan using specimens from broader range would provide more detailed knowledge about the relationships between the physicochemical environments and the spatial pattern of the biodiversity.

Tables

Table 2-1 The details of the components: number of stations included (*n*), average values of depth, temperature and salinity among sampling station within each component indicated by a letter assigned to each grid in Fig. 2-1 following the upper limit of the depth range.

Component	*N*	Mean environmental variables		
		Depth (m)	Temperature (°C)	Salinity (‰)
A150	4	179	5.2	34.1
A190	8	230	1.2	34.0
A300	2	340	0.5	34.1
B150	4	185	6.1	34.2
B190	32	230	3.1	34.1
B300	6	340	0.7	34.0
B400	4	452	0.5	34.0
C190	9	243	1.4	34.0
C300	11	336	0.7	34.0
C400	2	435	0.6	34.0
D190	20	255	2.0	34.0
D300	11	341	0.6	34.0
D400	5	448	0.4	34.0
E190	11	244	2.4	34.0
E300	6	342	0.8	34.0
E400	6	428	0.6	34.0

Table 2-2 Details of the samples used in this study: depth range of stations where *Lycodes* species were collected, range of total length of each *Lycodes* species (*TL*), number of specimens (*n*) and total wet weight of specimens (*W*).

Species	Depth range (m)	*TL* (mm)	*N*	*W* (kg)
L. tanakae	203–487	109–880	755	392
L. matsubarai	203–432	123–431	710	105
L. toyamensis	224–487	119–473	2377	318
L. nakamurai	204–487	80–451	3487	366
L. japonicus	293–442	100–156	114	1

L. teraoi	202–281	82–184	75	1
total			7518	1182

Table 2-3 Mean density of *Lycodes* species in each component.

Component	Mean density (no./ km^2)					
	L. tanakae	*L. matsubarai*	*L. toyamensis*	*L. nakamurai*	*L.japonicus*	*L. teraoi*
A150	0	0	0	0	0	0
A190	65	82	0	0	0	0
A300	70	3042	0	0	0	0
B150	0	0	0	0	0	0
B190	114	8	5	19	0	35
B300	443	306	1430	503	0	0
B400	92	0	1439	261	5	0
C190	145	2	172	772	0	17
C300	40	0	874	2039	2	0
C400	10	0	1082	361	0	0
D190	147	40	580	748	1	16
D300	166	524	661	504	128	6
D400	35	8	743	289	141	8
E190	32	0	27	118	0	4
E300	87	0	204	2288	14	0
E400	33	0	336	923	10	0

Table 2-4 Results of DistLM analyses on the species composition of *Lycodes*.

Variables	Pseudo-*F*	*P*	Proportion	Cumulative proportion
Depth	3.5	0.050	0.23	0.23
Temperature	6.9	0.008	0.30	0.52
Salinity	7.1	0.006	0.20	0.72

Figures

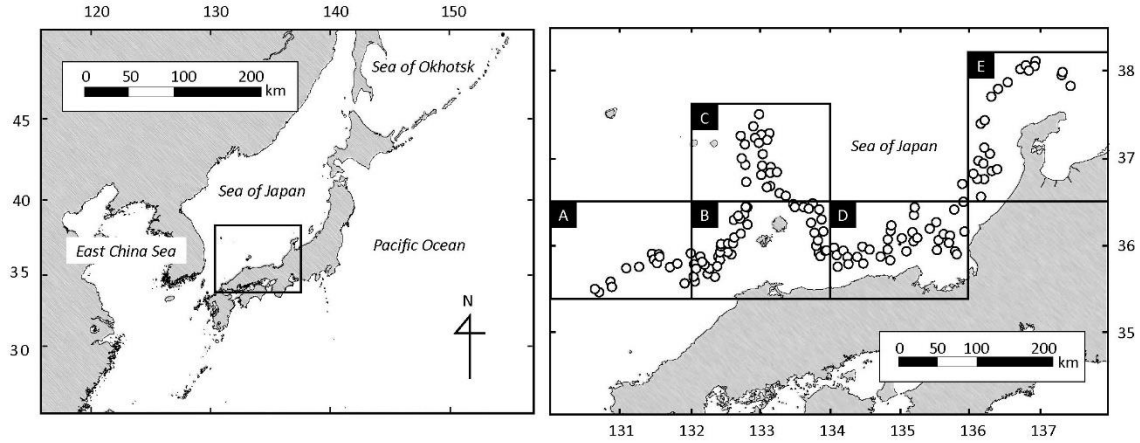

Fig. 2-1 Maps showing the study area in the southwestern Sea of Japan. Sampling stations were indicated by the small circles. The grouping of the stations for statistical analyses (A to E) was also shown.

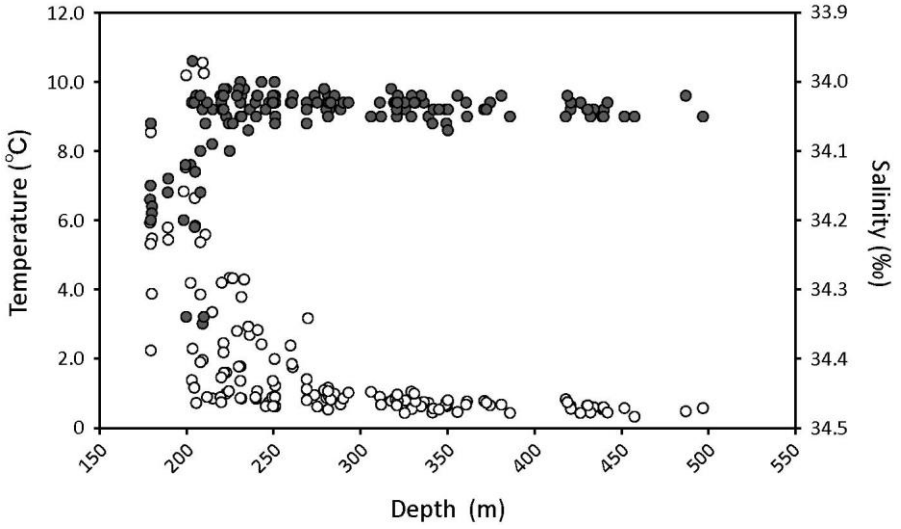

Fig. 2-2 Bathymetric changes of bottom water temperature (blank circles) and salinity (filled circles). Data from all trawl samplings conducted in this study were plotted.

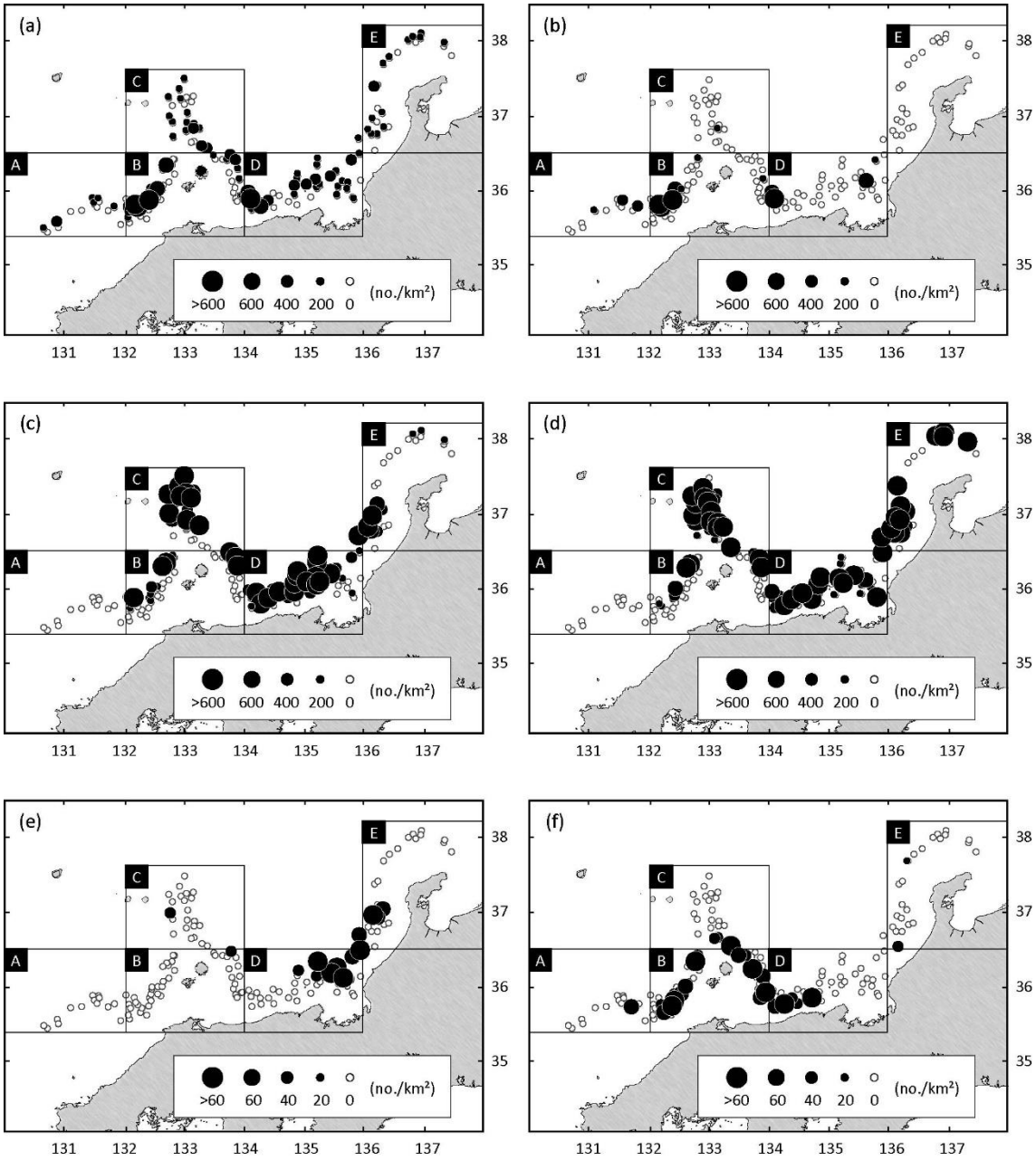

Fig. 2-3 Geographic distribution of *Lycodes* species in the western Sea of Japan coded by density (no./km^2): (a) *L. tanakae*; (b) *L. matsubarai*; (c) *L. toyamensis*; (d) *L. nakamurai*; (e) *L. japonicas;* (f) *L. teraoi*.

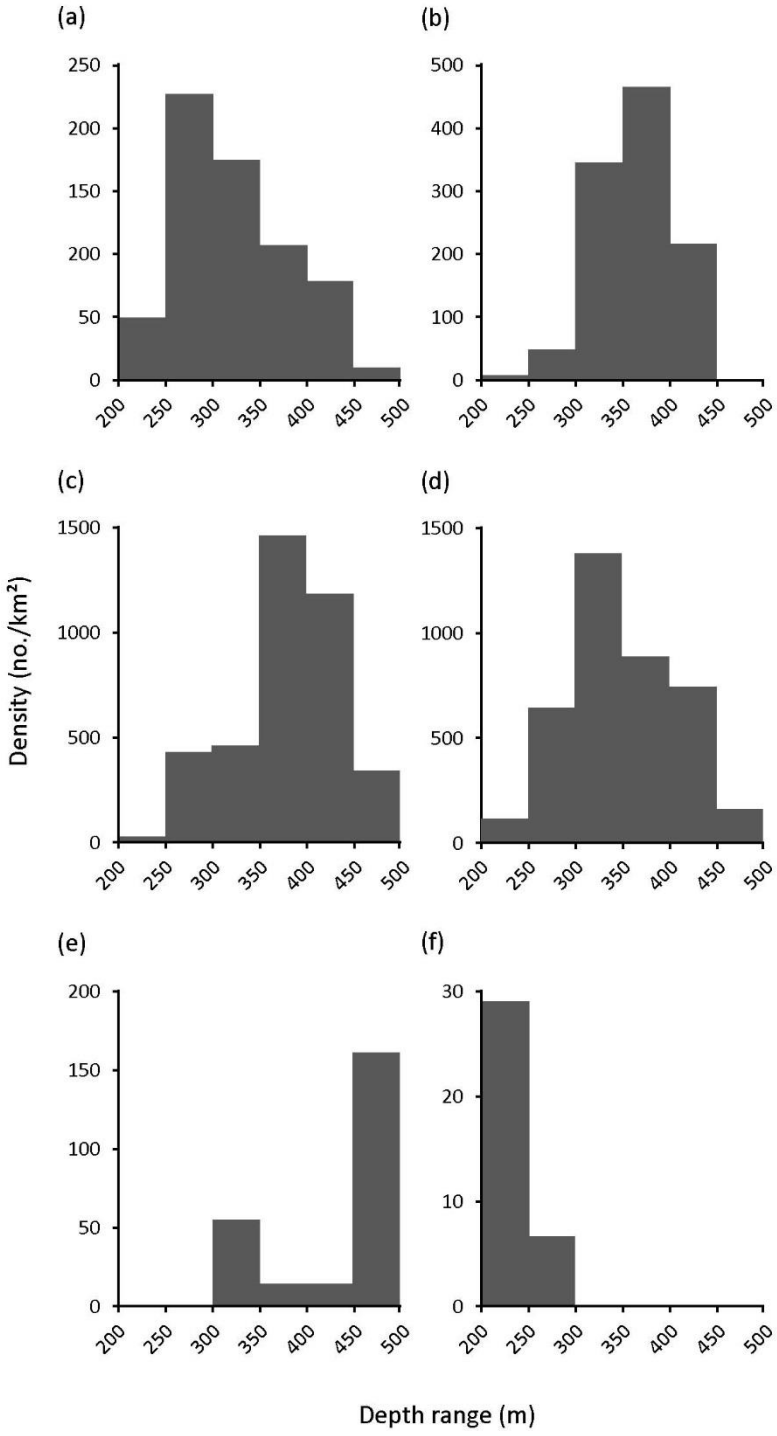

Fig. 2-4 Bathymetric distribution of *Lycodes* species shown by average density (no./km^2) within six depth ranges: (a) *L. tanakae*; (b) *L. matsubarai*; (c) *L. toyamensis*; (d) *L. nakamurai*; (e) *L. japonicas*; (f) *L. teraoi*.

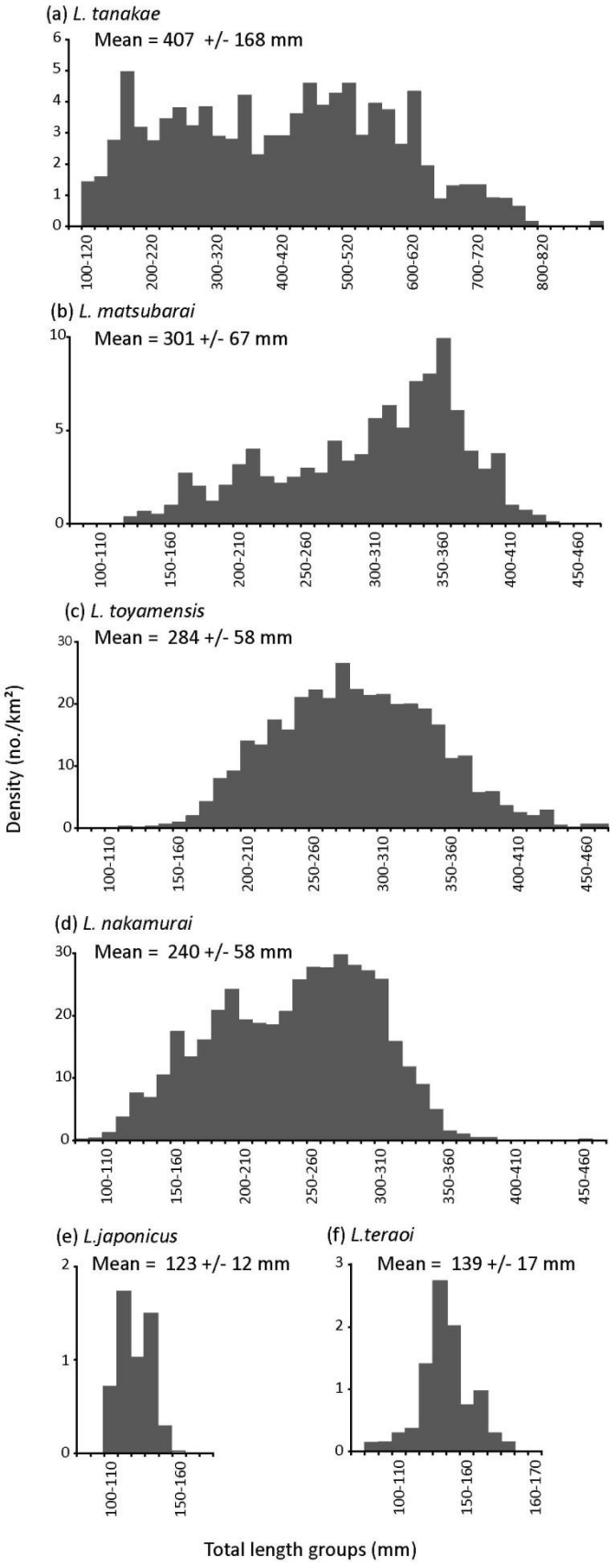

Fig. 2-5 Size frequency distribution of each *Lycodes* species shown by average density of all specimens (no./km^2): (a) *L. tanakae*; (b) *L. matsubarai*; (c) *L. toyamensis*; (d) *L. nakamurai*; (e) *L. japonicas*; (f) *L. teraoi*

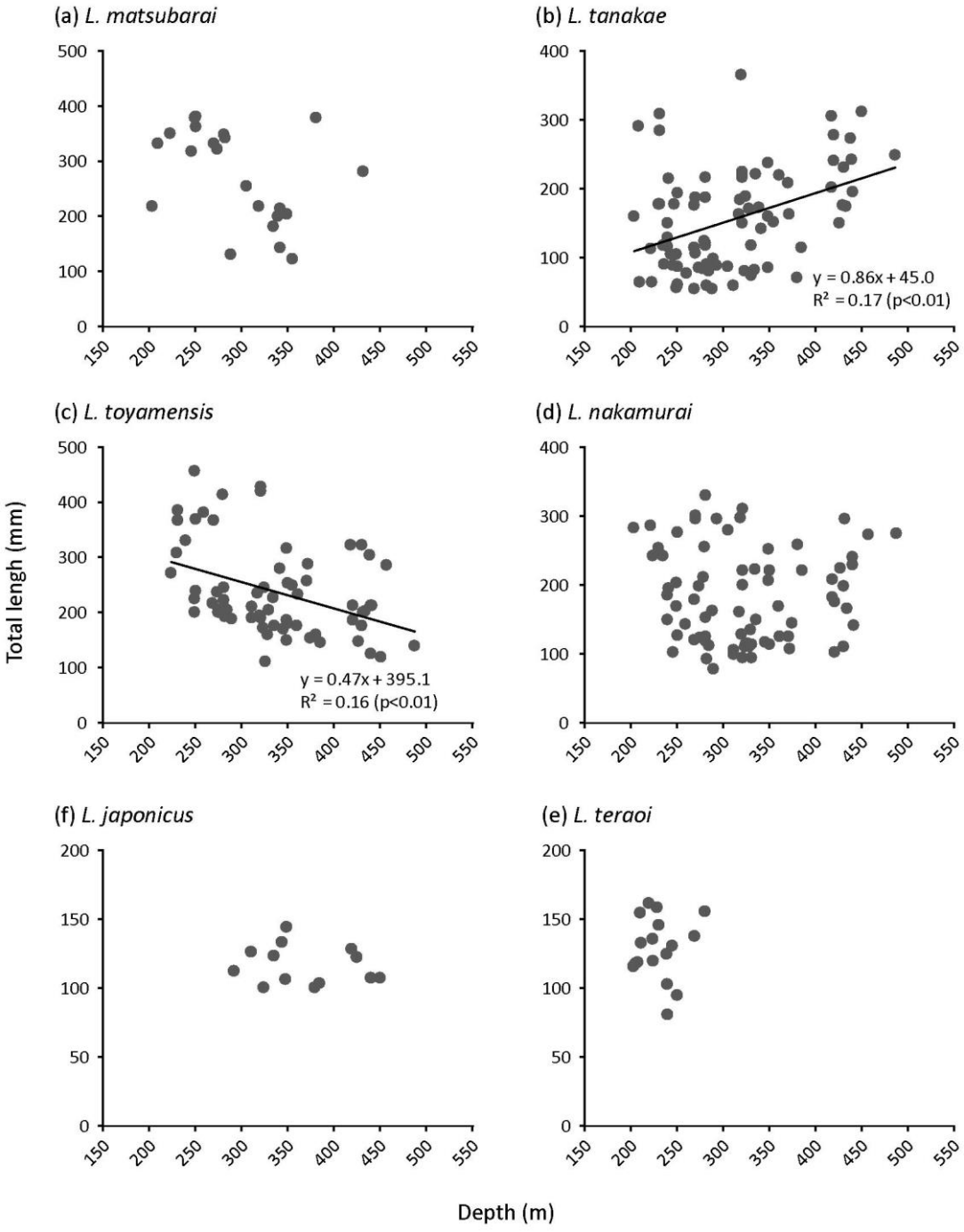

Fig. 2-6 Relationship between the depth of sampling station and the total length (TL) of the smallest individual of each *Lycodes* species obtained from each sampling station: (a) *L. tanakae*; (b) *L. matsubarai*; (c) *L. toyamensis*; (d) *L. nakamurai*; (e) *L. japonicas*; (f) *L. teraoi*

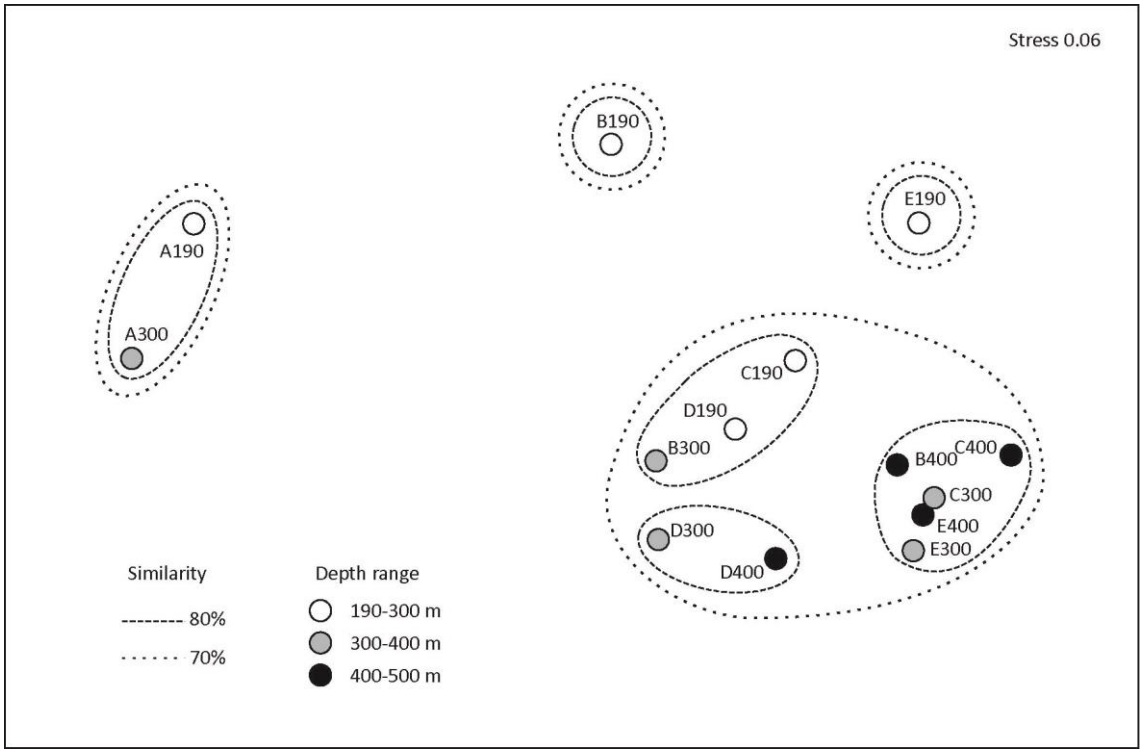

Fig. 2-7 The non-metric multi-dimensional scaling (nMDS) 2-dimensional plot showing the resemblances in species composition of *Lycodes* fishes among 16 components in the southwestern Sea of Japan. The groups recognized under the resemblances of 70% and 80% were shown by lines.

Chapter 3

Population genetics of *Lycodes matsubarai* in the Sea of Japan and the Sea of Okhotsk

3.1. Introduction

The population history of shallow-water organisms are, more or less, affected by the glacial climate changes during the Pleistocene. The previous studies have revealed that regional variation of such impacts have created glacial refugia, namely, the restricted local habitats that has been exempted from environmental changes. As I mentioned in General introduction, the glacial impacts on the local populations of deep-sea species are, however, still elusive.

In this chapter, I investigate the genetic variation of the Matsubara's eelpout, *Lycodes matsubarai* Toyoshima, 1985, a deep-sea demersal fish inhabiting the upper continental shelves in the Sea of Japan and the Sea of Okhotsk to compare population histories between the two sea areas (Fig. 3-1). The Sea of Japan and the Sea of Okhotsk are currently connected by shallow sea ways (i.e. the Soya Strait and the Tartar Strait) and thus the deep-sea habitats in these two sea areas are separated in present days. In addition, the fishes of the genus *Lycodes* lack pelagic egg and planktonic larva (Matarese et al. 1989; Balanov et al. 2006), which makes them ideal subjects for detecting unique demographic history in each marginal sea.

Here, I reconstruct the population history of *L. matsubarai* to investigate the impact of climate changes on deep-sea species during the last glacial period. To achieve the goal, I compare contemporary population structure between local populations in the two sea areas and estimate the time since their divergence. Then, the demographic history of each population since the divergence is reconstructed. My results reveal that the contrastive population history of deep-sea spices in two neighboring sea areas, which infer the regional scale influences of climate change acting upon the deep-sea species.

3.2. Materials and methods

3.2.1. Sample collection and laboratory procedures

In 2012, 90 specimens of *L. matsubarai*, 59 from three locations in the Sea of Japan (J-1 to J-3), and 31 from three locations in the southern Sea of Okhotsk (O-1 to O-3), were collected using an otter-trawl (Fig. 3-1 and Table 3-1). Small muscle tissues were removed from the specimens, immediately fixed in 99% ethanol, and frozen at -30°C prior to DNA extraction. Genomic DNA was extracted from each muscle tissue using a DNeasy Blood & Tissue extraction kit (Qiagen), according to the manufacturer's protocol.

DNA fragments of the mitochondrial control region (CR) were amplified by polymerase chain reaction (PCR) using the primers ProL (5′-CTA CCT CCA ACT CCC AAA GC-3′, Palumbi et al. 1991) and Phe39H (5′-GGT TCA TTT TAA CAT CTT CAG-3′) designed on the basis of the mitochondrial sequence of *Lycodes toyamensis* (GenBank Accession Number: NC004409). DNA fragments of the cyt*b* gene were amplified using the primers GluDG-L (5′-TGA CTT GAA RAA CCA YCG TTG-3′, Palumbi 1996) and CB3H (5′-GGC AAA TAG GAA RTA TCA TTC-3′, Palumbi 1996). The PCR protocol consisted of an initial denaturing step (94°C for 2 min), 30 cycles of denaturation (30 s at 94°C), annealing (1 min at 50°C for each locus), and extension (1.5 min at 72°C), followed by a final extension step (5 min at 72°C). PCR products were purified using ExoSAP-IT (United States Biochemical), and subsequently sequenced in both directions using a BigDye terminator cycle sequencing kit v3.1 (Applied Biosystems) and visualized on an ABI 3130xl Genetic Analyzer. Sequences were aligned using Clustal W implemented in the program package MEGA5 (Tamura et al. 2011). All unique sequences generated in this study were submitted to GenBank (accession numbers KF984205–KF984234: Table 3-2).

3.2.2. Data analyses of mitochondrial DNA sequences

To visualize the relationships between haplotypes, statistical parsimony networks were constructed using TSC v1.21 (Clement et al. 2000) with a connection limit of 99%. In the case of noncoding CR, deletion was treated as a fifth state. To detect genetic differentiation between the populations, pairwise *F*st values were estimated, and their significance was assessed after

10,000 permutations. An exact test of population differentiation based on haplotype frequencies in the populations was also conducted using 100,000 steps of a Markov chain; the first 10,000 steps were discarded as a burn-in (Raymond and Rousset 1995). These tests of population differentiation were performed using the program package Arlequin 3.5 (Excoffier and Lischer 2010). A sequential Bonferroni correction (Rice 1989) was applied for multiple tests. We estimated the genetic diversity of the populations based on three indices (*H*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversities) using Arlequin. Mismatch distributions were also analyzed by identifying significant departures from model estimates that assumed a sudden demographic expansion with 1,000 replicates using Arlequin.

We estimated several demographic parameters, including time since population divergence (*T*), the most recent common ancestors (TMRCA) of extant haplotypes observed in each population, the effective population size (*N*e) of each population since the divergence, and the temporal changes of *N*e, using coalescent theory-based analyses. Coalescent theory-based methods can produce accurate results even when sample sizes are low, and the statistical power increases with the number of loci included (Felsenstein 2006); therefore, we used both the CR and the cyt*b* sequence data in the subsequent analysis. To convert mutation-scaled estimates of the demographic parameters, species-specific mutation rates and durations of generation time were required. Although neither the CR nor cyt*b* mutation rate has been reported in zoarcid fishes, the rate of cyt*b* in sticklebacks, a related group of Zoarcidae (Miya et al. 2003), was available. We used the mutation rate of cyt*b* in three-spined sticklebacks, *Gasterosteus aculeatus*, which is 2.045% per My (Mäkinen and Merilä 2008). Using a single molecular clock is considered risky; therefore, following the recommendation of Hickerson and Cunningham (2005), we tested whether applying either two-fold higher or one-half lower rates (i.e., 4.0 and 1.0%, respectively) would change our conclusions. We assumed a generation time of six years based on data from the other *Lycodes* species (*L. raridens*, Balanov et al. 2006), and both two-fold higher and one-half lower values (i.e., three and twelve years, respectively) were used to test whether our conclusions were robust.

To estimate the time since divergence of the two populations, the Isolation with Migration program (IMa2, Hey 2010) was used. All runs of IMa2 were performed in 'Markov Chain Monte Carlo (MCMC)' mode under the following conditions: -l (number of genealogies

saved every 100 steps) 50,000 (i.e., 50,000,000 steps); -b (number of genealogies to be discarded as a burn-in) 5,000,000 (i.e., 10%); -n (number of heated chains) 40; -fg (geometric heating scheme); and –g1 0.975 –g2 0.75 (heating terms of the geometric increment model). Prior values of θ (mutation-scaled effective population size), t (mutation-scaled age of divergence), and m (mutation-scaled pairwise migration rates), were determined after several trial runs. Posterior distributions of the relative mutation rates were also obtained from the IMa model, and the rate of the CR was estimated using the relative rates with the highest posterior probability based on the rate of cyt*b*. We performed three replicate runs using different starting seeds and calculated the mean values of the demographic parameters.

We used another coalescent theory-based program, Migrate-n (Beerli and Felsenstein 2001; Beerli 2006), to investigate historical changes in the effective population size of the populations based on sequence data. We also estimated TMRCA of the populations in the Sea of Japan and the Sea of Okhotsk using Migrate-n. Runs were recorded every 1,000 steps out of a total of 50,000,000 steps of MCMC, with a burn-in of 5,000,000 steps (i.e., 10%). To improve the searching efficiency in MCMC, a static heating scheme with eight different temperatures (1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, and 1,000,000) was employed. Suitable upper boundaries for the prior distribution of θ (mutation-scaled effective population size/generations) and m (mutation-scaled migration rate) were decided after a number of experimental runs. The mutation rate of the CR was estimated by comparing Watterson's effective population size for each locus and the value over all loci by using the Migrate-n program. The results were averaged over four replicate runs using different starting seeds.

3.3. Results

3.3.1. Population structure, genetic diversity, and the results of mismatch distribution analysis

We determined the entire sequence of the mitochondrial CR (865 or 866 bp), and partial sequences of cyt*b* (793 bp), from 90 individuals of *L. matsubarai*. While the haplotype networks of CR and cyt*b* gene were different in their shapes (Fig. 3-2), the haplotypes shared between the

Sea of Japan and the Sea of Okhotsk populations were consistently not present (CR) or rare (cyt*b*). The result was also concordant for two loci in the point that some "minor" haplotypes from each sea were excluded from the main, monophyletic group (i.e., CR09, CR11, CB03, and CB08). In addition to these points, haplotypes from the Sea of Japan showed clear signatures of recent demographic expansion in the cyt*b* network (i.e., a star phylogeny; Slatkin and Hudson 1991).

For the CR sequence data, pairwise *F*st values between sampling locations from the different seas ranged from 0.39 to 0.70, and the values were significantly different from zero for comparison pairs except for the pair of locations J-1 and O-2. Pairwise *F*st values between sampling locations from the same sea were small and not significantly different from zero (Table 3-2). For the cyt*b* sequence data, pairwise *F*st values ranged from 0.67 to 0.85, and the values were significantly different from zero for all nine comparison pairs of the sampling locations from the different seas after a sequential Bonferroni correction. *F*st values between sampling locations from the same sea were small and not significantly different from zero, including from the cyt*b* sequence data. Results from the exact test of population differentiation also showed significant genetic differentiation between the pairs of locations from the different seas, except for one comparison pair at locations O1 and J3 based on the cyt*b* sequence data. In contrast, the comparison pairs of sampling locations from the same sea were not significantly different. As we could not detect any trace of significant genetic differentiation within the same sea, individuals from the same sea were treated as single local populations (i.e., the Sea of Japan and the Sea of Okhotsk populations) in the subsequent analyses.

Genetic diversity indices, based on the CR and cyt*b* sequence data, were higher for the samples from the Sea of Okhotsk than for samples from the Sea of Japan (Table 3-3). The higher genetic diversity of the Sea of Okhotsk samples indicates a larger historical effective population size there than in the Sea of Japan (McCusker and Bentzen 2010).

From the results of the mismatch distribution analysis on the cyt*b* and CR sequence data, the sudden expansion model was not rejected for samples from the Sea of Japan (Fig. 3-3), which suggests that *L. matsubarai* experienced sudden population growth in the Sea of Japan. However, significant departures from the model estimates were seen in the samples from the Sea of Okhotsk based on the cyt*b* sequence data, whereas the sudden expansion model was not

rejected based on the CR data.

3.3.2. Time since the divergence of populations.

We obtained estimates for t (mutation-scaled time since divergence) and θ (mutation-scaled effective population size) for the Sea of Japan and the Sea of Okhotsk populations of *L. matsubarai* based on the IMa model. The mutation rate of the CR was estimated to be 1.3% per My. Estimates for θ and t were converted into N_e (effective population size) and T (time since divergence, in calendar years), respectively, by applying the geometric mean of the mutation rates and a generation time of six years (Figs. 3-4a and b). When we assumed the same mutation rates and generation times for *L. matsubarai* in the two seas, the long-term, mean effective population size in the Sea of Okhotsk since the divergence was at least two-fold larger than that in the Sea of Japan (Fig. 3-4a). The time since the divergence of the populations was estimated to be 20,000 years (95% highest posterior density intervals = 9,000–46,000 years; Fig. 3-4b).

3.3.3. Historical changes in effective population size

Skyline plots (Fig. 3-5) were constructed based on four independent runs of Migrate-n. The mutation rate of the CR was estimated to be 0.9% per My using Watterson's effective population size, which was slightly slower than the rate derived from the IMa model. Migrate-n crudely estimates the relative rates among loci based on the genetic diversity and the rate derived from the IMa model seems to be closer to the true value. TMRCA of the extant haplotypes from each sea existed during the middle stages of the last glacial period (ca. 38,000 years ago; Fig. 3-5). The effective population size in the Sea of Okhotsk was consistently larger than that in the Sea of Japan except for in the last few hundred years, and this result was concordant with that derived from the IMa model (Fig. 3-4a). The population size of *L. matsubarai* in the Sea of Okhotsk was stable or gradually increasing during the last glacial period, but suddenly decreased after the last glacial period. However, the population size in the

Sea of Japan continuously decreased during the last glacial period and then subsequently expanded.

3.4. Discussion

3.4.1. Population divergence of L. matsubarai *between the two seas*

The results from the sequence analyses of the non-coding CR and the protein-coding cyt*b* gene in the mitochondrial DNA clearly showed genetic divergence between the populations of *L. matsub*n*arai* in the Sea of Japan and the Sea of Okhotsk, which may be attributed to geographic isolatio during previous glacial periods. The Sea of Japan is connected to the Sea of Okhotsk by two shallow straits (Soya Strait and Tartar Strait: 55 and 13 m depth, respectively). Miller et al. (2005) reported that the global sea level fell approximately 130 m below the present level during the LGM, which strongly suggests that these straits became a land bridge during this period. In general, deep-sea fishes with a planktonic larval stage are widely distributed and tend to be genetically homogeneous over their distribution range, probably because of their high dispersal ability (White et al. 2011; Friess and Sedberry 2011). Adachi et al. (2009) found no evidence for the genetic divergence of blackfin sculpins, *Malacocottus* spp., among the populations in the Sea of Japan, Sea of Okhotsk, and Pacific Ocean. In contrast, regional populations are likely to diverge from each other in species with low dispersal ability (Kai et al. 2011). As mentioned above, *L. matsubarai* lacks pelagic eggs and planktonic larvae and its genetic exchange is attributed to adult dispersal; therefore, post-glacial migration is thought to have been restricted by the shallow strait.

The divergence age estimates based on the IMa model suggest that the populations of the two seas diverged during the last glacial period, probably because of geographic isolation between the Sea of Japan and the Sea of Okhotsk due to the formation of the Soya land bridge (Figs. 3-6a and b). Even if a range of mutation rates (1.0–4.0% per My) is considered, it can still be assumed that populations in the Sea of Japan and the Sea of Okhotsk diverged during the last glacial period (Figs. 3-6a and b). From these results, we can assume that the population in each

sea has had an independent history since the last glacial period, which should be reflected in the different demographic parameters of the populations.

The shapes of the haplotype networks were different between two mitochondrial loci (Fig. 3-2). Such a difference might be attributed to the random disappearance of haplotypes and nucleotide substitutions in each population, which have independently occurred in two loci. Although the two populations were statistically different from each other, some haplotypes in each population were excluded from the major monophyletic group in the networks of the two loci. This might suggest incomplete lineage sorting due to the short period since the divergence, or occasional dispersal after the divergence. Unfortunately, we cannot determine whether they are ancestral polymorphisms or post-divergence migrants between the two seas because the present results are based on only two mitochondrial loci, which are linked to each other and can only provide rough estimates of the demographic parameters. We also estimated temporal changes in the number of migrants between the two seas using the Migrate-n program (data not shown), but could not interpret the results as the range of standard errors was too large.

3.4.2. Demographic history of L. matsubarai *in the two seas*

Historically, the population of *L. matsubarai* in the Sea of Japan has had a smaller effective population size than that in the Sea of Okhotsk, except for the last few hundred years (Figs. 3-4a and 3-5). The star-shaped phylogeny of haplotype networks (Fig. 3-2) and the results of the mismatch distribution analysis (Fig. 3-3) strongly suggest that the population in the Sea of Japan has recently expanded. The skyline plot showed that the population in the Sea of Japan experienced a genetic bottleneck during the last glacial period and recovered subsequently (Fig. 3-6c). During the LGM, density stratification in the water column developed in the Sea of Japan, which resulted in fatally anoxic sea-bottom conditions in a large area of this sea (Itaki et al. 2004). Some deep-sea species in the Sea of Japan may therefore have experienced a rapid increase in population size after the last glacial period (Shirai et al. 2006; Adachi et al. 2009). The present results support the hypothesis that glacial periods have had a significant impact on deep-sea organisms in the Sea of Japan.

The population of *L. matsubarai* in the Sea of Okhotsk has had a larger effective population size than that in the Sea of Japan during most periods since divergence (Figs. 3-4a and 3-5). The higher values of the genetic diversity indices suggest that the population of *L. matsubarai* flourished under suitable conditions in the Sea of Okhotsk during the last glacial period. The cyt*b* sequence data showed significant departures from the estimates from the sudden expansion model for the Sea of Okhotsk population, which suggests that this population has not experienced any recent population expansion. Although the southern part of the Sea of Okhotsk was covered by seasonal sea ice during the last glacial period (Shiga and Koizumi 2000; Sakamoto et al. 2005), the bottom did not became anoxic even during the LGM (Gorbarenko et al. 2004), which likely enabled *L. matsubarai* to maintain a stable population there.

The skyline plot for the population in the Sea of Okhotsk clearly showed the signature of a recent population reduction, which occurred synchronously with the population expansion in the Sea of Japan. Applying a range of mutation rates (1.0–4.0% per My) and generation times (3–12 years) did not change my result, which indicates that the abrupt changes in effective population size postdate the end of the last glacial period in both seas (Figs. 3-6a and b). Okazaki et al. (2005) reported that the present oceanic regime in the southern Sea of Okhotsk was established 8,000 years ago. In the Sea of Japan, the present oceanic conditions have existed for the last 8,000 years (Ishiwatari et al. 2001). Such changes in oceanic conditions followed global trends in climate (Imbrie et al. 1989); hence, it might be reasonable to infer that the changes in the oceanic regime in the Sea of Okhotsk and the Sea of Japan are tightly linked to each other. We suggest that the synchronous changes in the effective population sizes of *L. matsubarai* in the two seas were closely related to the global changes in climatic and oceanic conditions induced by glacial cycles.

Incomplete sampling is a possible source of uncertainty in population genetic studies, which may have led to an underestimation of the population age (Ruzzante et al. 2008). In addition to the smallness of sampling sites (three locations in each sea), our sampling locations did not fully cover the geographic range of *L. matsubarai* in the Sea of Japan, as *L. matsubarai* has also been recorded on the Yamato Bank, located in the central part of the sea (Okiyama, 2004). Unfortunately, we could not obtain enough samples for genetic analyses from this area,

and it is possible that additional samples would have influenced our conclusion. Therefore, exhaustive sampling that fully covers the study area is required to confirm the conclusions of this study.

3.4.3. Contrasting population histories of deep-sea fish in the two neighboring seas

Although recent population expansions may have occurred in various marine fishes (Avise 2000), the mechanisms driving the changes in effective population size need to be investigated individually, as local populations of marine species have independent population histories that are controlled by different driving forces. In addition, far fewer reports have been published on the population genetics of deep-sea species than those of shallow-water species. Varela et al. (2012) suggested that glacial-interglacial cycles and associated changes in the marine environment affected the demographic history of the orange roughy, *Hoplostethus atlanticus*. Although demographic expansion has also been reported for *Beryx decadactylus* (Friess and Sedberry 2011) and *Aphanopus carbo* (Sergio and Knutsen 2007), the causes of these demographic changes have not been discussed. In the case of *L. matsubarai*, a regional difference in deep-sea ichthyofauna between the two seas may be informative in terms of its population history. A limited number of deep-sea fish species (approximately 20) inhabit the Sea of Japan, which is characterized by low, patchy, and seasonal surface productivity (Zenkevitch 1963; Tyler 2002; Okiyama 2004). In contrast, the Sea of Okhotsk is one of the most productive seas in the world (Mordasova 1997), and more than 80 fish species have been reported from the deep-sea area (Savin 2012). The present data do not allow us to discuss why the effective population size has decreased in the Sea of Okhotsk; however, the interspecific competition associated with the high species diversity of deep-sea fishes in this area might be related to this phenomenon. In any case, the results clearly showed that regional-scale variation in responses to climate change exists, even in a single species.

In conclusion, our data showed that populations of a deep-sea fish, *L. matsubarai*, in the Sea of Japan and the Sea of Okhotsk experienced contrasting demographic histories, which are likely to postdate the divergence of the two populations during the last glacial period. The

two neighboring seas had different oceanographic conditions during the last glacial period, and the deep-sea ichthyofauna of the two seas also differed. These circumstances may have affected the demographic changes of each population after the last glacial period. The present results indicate that the population histories of deep-sea species can vary, even at the regional scale. The population histories of deep-sea fish inhabiting the marginal seas of the northwestern Pacific Ocean require further investigation in order to elucidate the effects of climate change during the glacial cycles.

Tables

Table 3-1 Sampling date, location, and number of samples of *Lycodes matsubarai* used in this study. The names of the vessels and their affiliation are also provided.

Samples	Date	Latitude	Longitude	Depth (m)	*n*	Vessels
Sea of Japan (SOJ)						
J-1	May 2012	35˚51.38 N	132˚35.20 E	341	16	T/S Tanshu-Maru, Hyogo pref.
J-2	May 2012	35˚53.23 N	131˚28.41 E	344	20	T/S Tanshu-Maru, Hyogo pref.
J-3	May 2012	35˚53.10 N	131˚34.29 E	342	23	T/S Tanshu-Maru, Hyogo pref.
total					59	
Sea of Okhotsk (OKH)						
O-1	Apr 2012	45˚06.24 N	143˚37.48 E	163	12	Daigo-Kaiyo-Maru, Nippon Kaiyo Co., Ltd.
O-2	Apr 2012	44˚43.12 N	143˚46.54 E	155	4	Daigo-Kaiyo-Maru, Nippon Kaiyo Co., Ltd.
O-3	Apr 2012	44˚28.36 N	144˚06.00 E	212	15	Daigo-Kaiyo-Maru, Nippon Kaiyo Co., Ltd.
total					31	

.

Table 3-2 Nucleotide variation and frequencies of the mitochondrial control region (CR: a) and cytochrome *b* gene (cyt*b*: b) haplotypes of *Lycodes matsubarai*. SOJ and OKH represent the Sea of Japan and the Sea of Okhotsk, respectively.

(a)

Haplotype	GenBank Accession numbers	Nucleotide position from 5' end									Number of individuals from each sampling areas	
		21	32	55	144	276	468	704	708	865	SOJ	OKH
CR01	KF984205	G	G	G	T	G	T	A	G	A	0	19
CR02	KF984206	.	A	.	.	.	.	.	.	.	0	7
CR03	KF984207	.	.	.	.	.	.	.	.	T	0	1
CR04	KF984208	.	.	A	*	A	T	.	.	.	0	2
CR05	KF984209	.	.	.	.	.	C	.	.	.	0	1
CR06	KF984210	.	A	.	*	A	T	.	.	.	0	1
CR07	KF984211	.	A	.	.	.	T	T	.	.	48	0
CR08	KF984212	.	A	.	.	.	C	T	.	.	2	0
CR09	KF984213	.	A	.	*	A	C	.	.	.	3	0
CR10	KF984214	.	A	.	.	.	T	T	A	.	2	0
CR11	KF984215	.	A	A	.	A	T	.	.	.	3	0
CR12	KF984216	A	A	.	.	.	.	T	.	.	1	0

(b)

Haplotype	GenBank Accession numbers	Nucleotide position from 5' end																		Number of individuals from each sampling areas	
		28	72	144	154	248	275	288	405	408	441	444	450	459	588	616	654	663	766	SOJ	OKH
CB01	KF984217	A	G	T	G	T	T	G	G	G	G	G	C	C	G	A	T	G	T	0	9
CB02	KF984218	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	0	5
CB03	KF984219	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	1	5
CB04	KF984220	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	C	.	.	0	1
CB05	KF984221	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A	.	0	1
CB06	KF984222	.	.	.	.	.	.	.	.	.	.	A	T	.	A	.	.	.	.	0	1
CB07	KF984223	.	A	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	C	0	1
CB08	KF984224	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	C	51	1
CB09	KF984225	.	A	.	A	.	.	.	.	.	A	.	.	T	.	.	.	.	C	0	1
CB10	KF984226	.	.	.	.	.	.	.	A	.	.	.	T	.	A	.	.	.	.	0	1
CB11	KF984227	G	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	0	1
CB12	KF984228	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	2
CB13	KF984229	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	0	1
CB14	KF984230	.	.	.	.	.	.	C	.	.	.	A	.	T	A	.	.	.	C	0	1
CB15	KF984231	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	C	3	0
CB16	KF984232	.	.	.	.	C	.	.	.	.	A	.	.	T	.	.	.	.	C	2	0
CB17	KF984233	.	.	.	.	.	C	.	.	.	A	.	.	T	.	.	.	.	C	1	0
CB18	KF984234	.	.	.	.	.	.	.	.	.	A	.	.	T	.	G	.	.	C	1	0

Table 3-3 Results of the tests of pairwise *F*st values (below the diagonal) and the exact tests of population differentiation based on haplotype frequencies (above the diagonal) for *Lycodes matsubarai* sampled from three locations in the Sea of Japan (J-1 to J-3) and three locations in the Sea of Okhotsk (O-1 to O-3) using the mitochondrial control region (CR: a) and cytochrome *b* (cyt*b*: b) sequences. Significant *F*st values and *P* values ($P < 0.05$) are indicated by bold numerals and asterisks, respectively.

(a)

Samples	J-1	J-2	J-3	O-1	O-2	O-3
J-1	-			*	*	*
J-2	0.01	-		*	*	*
J-3	0.00	-0.01	-	*	*	*
O-1	**0.55**	**0.70**	**0.62**	-		
O-2	0.39	**0.64**	**0.51**	-0.01	-	
O-3	**0.53**	**0.67**	**0.60**	-0.05	-0.04	-

(b)

Samples	J-1	J-2	J-3	O-1	O-2	O-3
J-1	-			*	*	*
J-2	-0.01	-		*	*	*
J-3	-0.03	-0.01	-		*	*
O-1	**0.73**	**0.67**	**0.68**	-		
O-2	**0.85**	**0.77**	**0.79**	0.06	-	
O-3	**0.78**	**0.71**	**0.72**	-0.01	-0.02	-

Table 3-3 Number of individuals (*N*), number of haplotypes (*H*), mean haplotype diversity (*h*), and mean nucleotide diversity (π) of *Lycodes matsubarai* at each sampling location. The values for each sea are also shown.

Samples	*N*	*D-loop*			*Cytochrome b*		
		H	*h*	π	*H*	*h*	π
Sea of Japan (SOJ)							
J-1	16	3	0.34	0.0013	5	0.45	0.0006
J-2	20	4	0.28	0.0006	2	0.10	0.0008
J-3	23	4	0.39	0.0010	3	0.25	0.0003
total	**59**	**6**	**0.34**	**0.0010**	**6**	**0.25**	**0.0005**
Sea of Okhotsk (OKH)							
O-1	12	4	0.56	0.0011	7	0.89	0.0034
O-2	4	3	0.83	0.0019	4	1.00	0.0044
O-3	15	4	0.60	0.0011	8	0.83	0.0032
total	**31**	**6**	**0.58**	**0.0012**	**14**	**0.88**	**0.0034**

Figures

Fig. 3-1 *Lycodes matsubarai* collected at the station J-1 in the Sea of Japan.

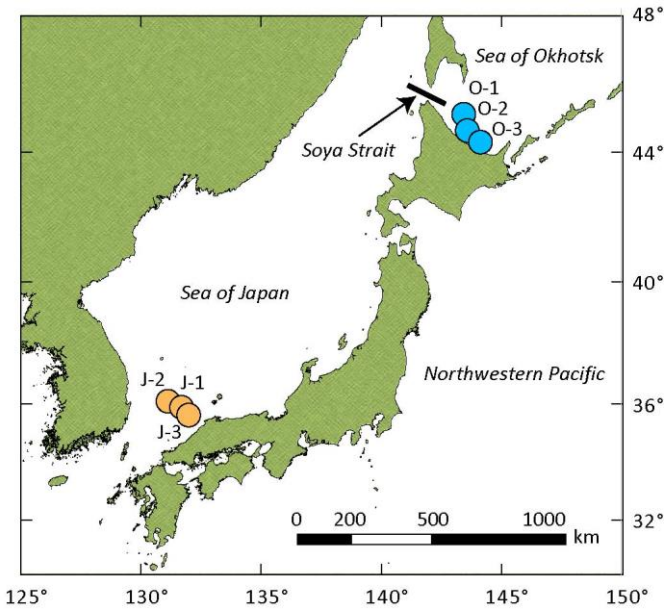

Fig. 3-2 Map of the study area, showing sampling locations in the Sea of Japan (open circles) and in the Sea of Okhotsk (filled circles).

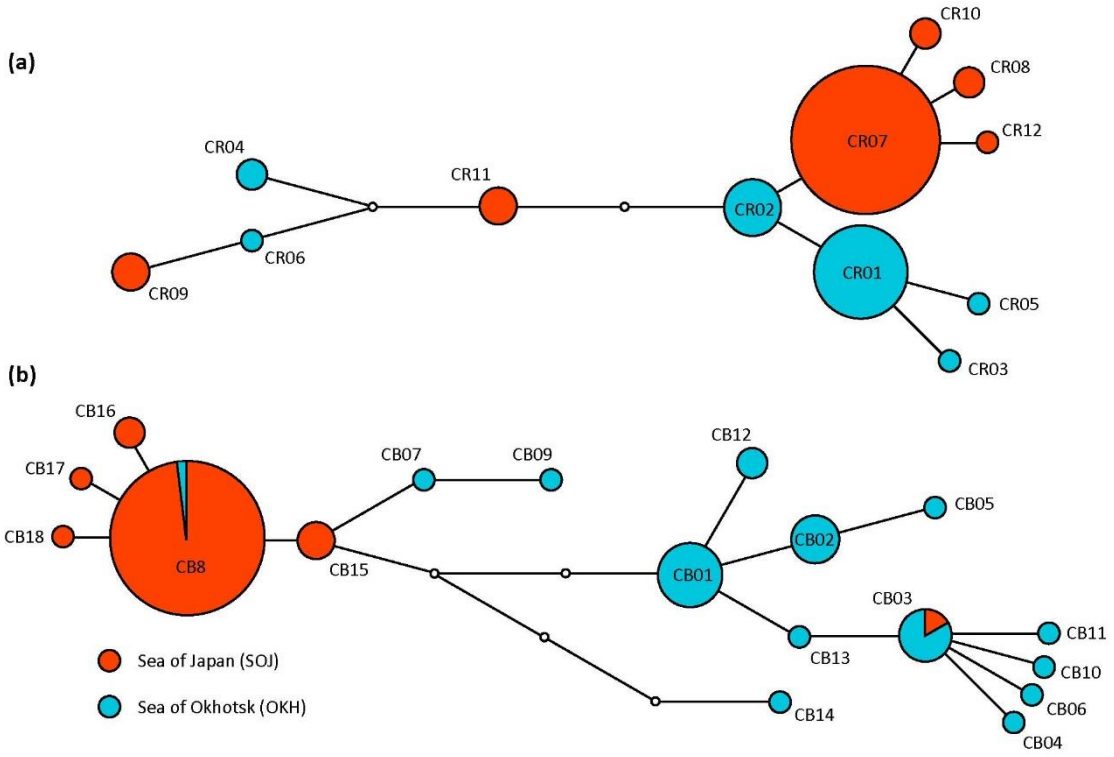

Fig. 3-3 Statistical parsimony haplotype networks of *Lycodes matsubarai*, based on nucleotide sequences of the mitochondrial control region (CR: a) and the cytochrome *b* gene (cyt*b*: b). Circle size is proportional to the number of individuals of each haplotype, and labels indicate the names of haplotypes. Small open circles indicate intermediate haplotypes that were not sampled.

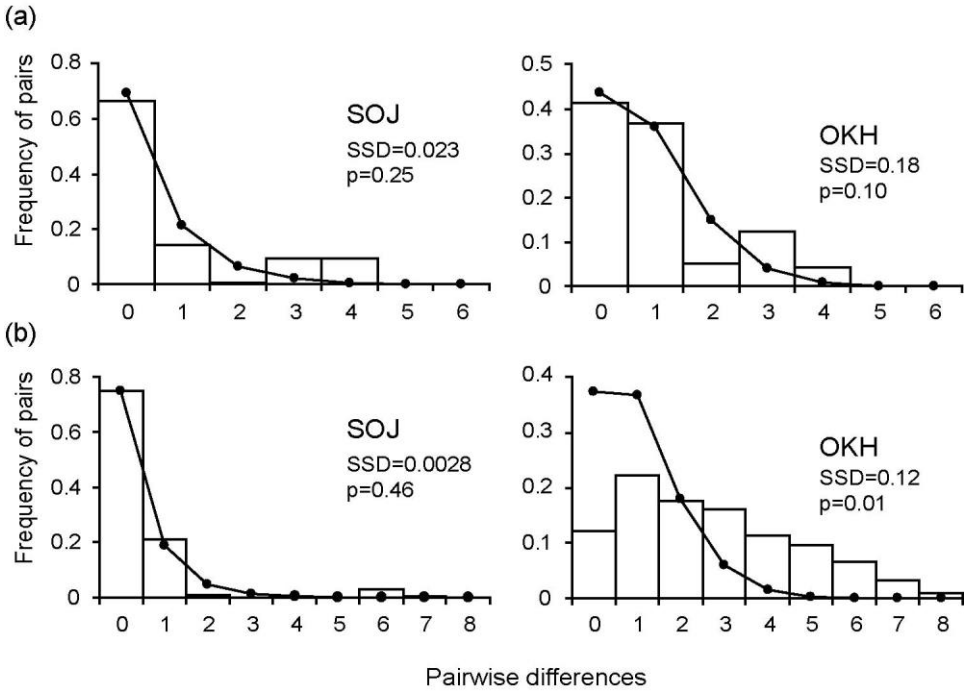

Fig. 3-4 Mismatch distributions based on nucleotide sequences of the mitochondrial control region (CR: a) and the cytochrome *b* (cyt*b*: b) gene of *Lycodes matsubarai*. Lines represent the expected frequencies of mismatches under the sudden expansion model. SOJ and OKH represent the samples from the Sea of Okhotsk and the Sea of Japan, respectively. SSD indicates the sum of square deviations between the observed frequencies and estimates based on the sudden expansion model, and *P*-values indicate the significance of the tests for goodness of fit.

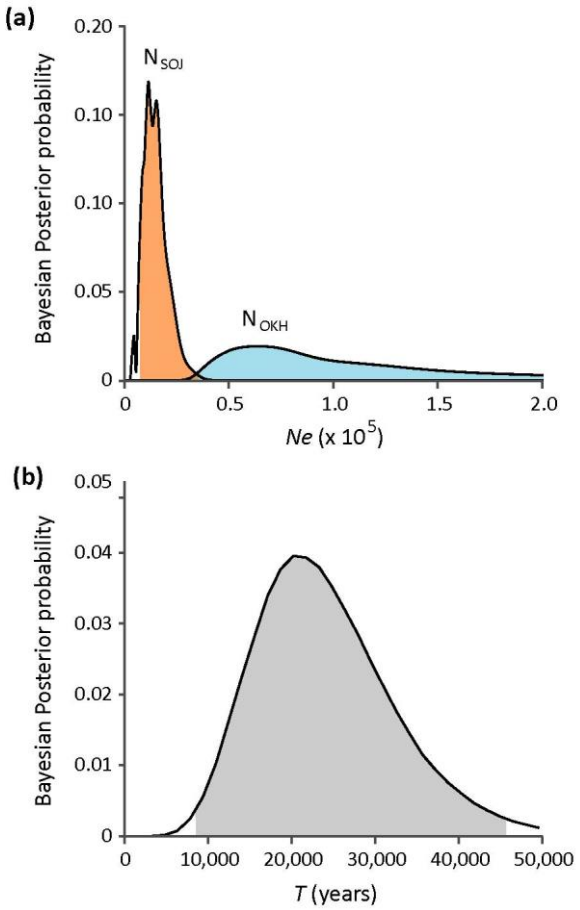

Fig. 3-5 Posterior distribution of estimates from the Isolation with Migration model on combined data from the mitochondrial control region (CR) and the cytochrome *b* (cyt*b*) sequences. Shaded areas show 95% highest posterior density intervals. (a) Effective population size of *Lycodes matsubarai* in the Sea of Japan (N_{SOJ}), and in the Sea of Okhotsk (N_{OKH}). (b) Time since divergence (*T*) of the populations in the two seas.

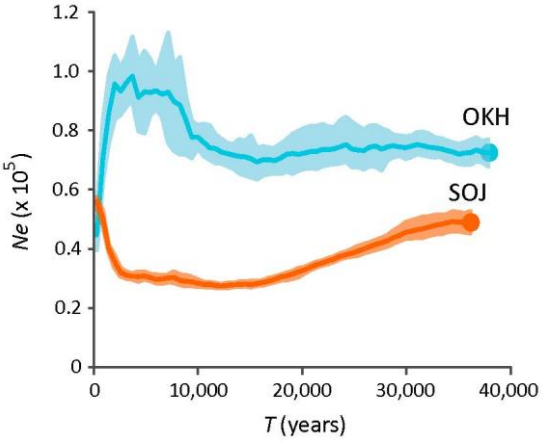

Fig. 3-6 Skyline plots illustrating demographic changes in the Sea of Japan and the Sea of Okhotsk populations of *Lycodes matsubarai*. Solid circles show times to the most recent common ancestor of sampled haplotypes. Lines indicate the median estimates among four independent runs of Migrate-n. Shaded areas show 95% highest posterior density intervals, considering all four runs. SOJ and OKH represent the populations in the Sea of Okhotsk and in the Sea of Japan, respectively.

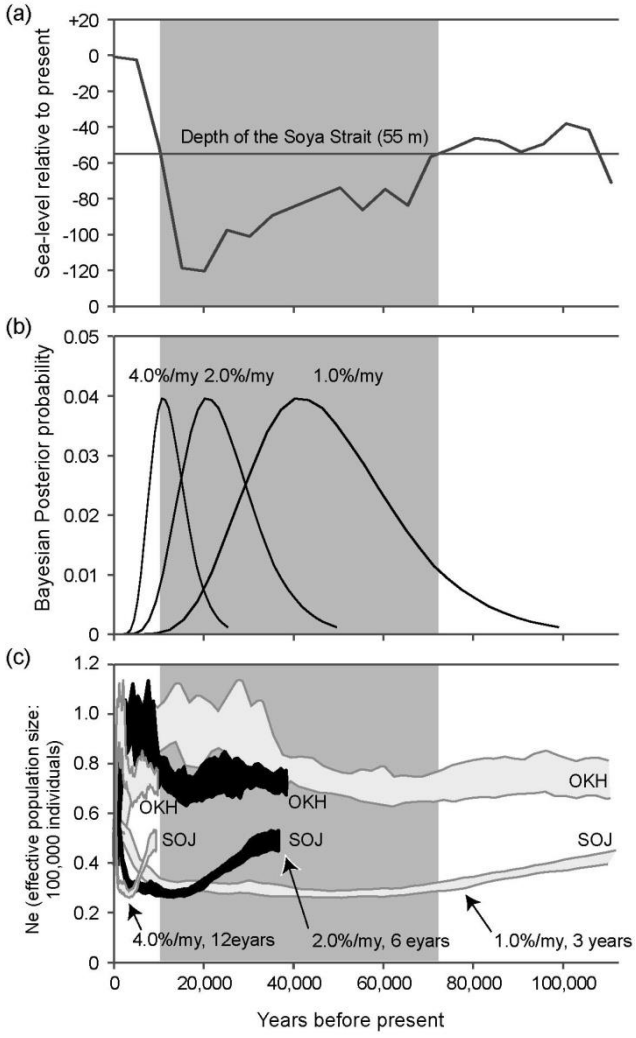

Fig. 3-7 (a) Global trends in sea level change during the last 100,000 years (modified from Miller et al. 2005). A shaded area indicates the duration that the sea level was lower than the present depth of the Soya Strait (55 m). (b) Posterior distribution of time since the divergence (*T*) under the Isolation with Migration model, and the range of mutation rates (1.0–4.0% per My in cytochrome *b*). (c) Skyline plots illustrating demographic changes in the Sea of Japan and the Sea of Okhotsk populations of *Lycodes matsubarai*, under a range of mutation rates (1.0–4.0% per My in cytochrome *b*) and generation times (3–12 years).

Chapter 4

Population genetics of *Lycodes japonicus* in the Sea of Japan and *L. ocellatus* in the northwestern Pacific Ocean

4.1. Introduction

The modes in diversification processes of deep-sea fishes are elusive. As I mentioned in Chapter 1, few studies have succeeded in the elucidating the causes that triggered the speciation event. In this chapter, I examined the speciation process of two *Lycodes* species: *L. japonicus* Matsubara and Iwai, 1951 and *L. ocellatus* Toyoshima, 1985 (Fig. 4-1). These species are endemic to the upper continental shelves around the Japanese Archipelago: *L. japonicus* is endemic to the Sea of Japan, and *L. ocellatus* has been reported only from the Pacific Ocean off the northeastern part of Japan. These two species show similar body coloration (Fig. 4-1; Table 4-1), and the ranges of meristic characters overlap considerably (Table 4-1). In Chapter 1, I also showed the sister relationships of the two species by the molecular phylogenetic analysis.

The distribution ranges of *L. japonicus* and *L. ocellatus* are currently separated by the Tsugaru Strait (130 m depth), which may have played an important role in the speciation process. Previous studies have pointed out the roles of sea-level minima during the last glacial period, which isolated the Sea of Japan from neighboring sea areas and induced allopatric divergence of deep-sea populations of the Japan Sea eelpout, *Bothrocara hollandi* (Kojima et al. 2001), however, the age of the speciation event was not estimated based on a reliable dataset.

Here, I investigated the population genetic structure of *L. japonicus* and *L. ocellatus* to confirm the phylogenetic relationships of these two species and estimated the time since divergence under Bayesian inference. The demographic history of the two species was also reconstructed to determine the impacts of environmental changes in the Sea of Japan during the last glacial period (Itaki et al. 2004; Gorbarenko and Southon 2000). It has been suggested that expansion of persistent low oxygen zones have restricted connectivity among local populations and promote speciation in the northern Pacific (Hyde and Vetter 2007).

The aim of my study was to evaluate the roles of drastic climate changes during the mid- to late Pleistocene in the speciation of two *Lycodes* species in the northwestern Pacific

areas. My results shed light on the role of climatic oscillation in shaping the current diversity of the genus *Lycodes*, and may help elucidate the diversification process of deep-sea fishes in the northern Pacific marginal seas.

4.2. Materials and methods

4.2.1. Sample collection and laboratory procedures

I collected 53 samples of *L. japonicus* from 2 sites in the Sea of Japan (J-1, J-2) and 37 samples of *L. ocellatus* from 3 sites in the northwestern Pacific Ocean (O1–O3), during trawl surveys conducted by Fisheries Research Agency of Japan (Fig. 4-1 and Table 4-2).

Small muscle tissues were removed from each specimen, immediately placed into 99% ethanol on board, and stored in a freezer (–30°C) prior to DNA extraction. Genomic DNA was extracted using a DNeasy Blood & Tissue extraction kit (Qiagen) following the manufacturer's protocol. DNA fragments of the mitochondrial COI were amplified using the universal primers LCO1490 and HCO2198, and those of cyt*b* were amplified using the primers GluDG-L and CB3H. The primers used here were listed in the Chapter 1. The PCR conditions comprised an initial denaturing cycle (94°C for 2 min), 30 cycles of denaturation (30 s at 94°C), annealing (1 min at 47°C for COI and 50°C for cyt*b*), and extension (1.5 min at 72°C), followed by a final extension step (5 min at 72°C). PCR products were directly purified using ExoSAP-IT (United States Biochemical), subsequently sequenced in both directions using a BigDye terminator cycle sequencing kit version 3.1, and visualized on an ABI 3130xl Genetic Analyzer. Sequences were aligned using Clustal W implemented in the program package MEGA5, manually checked, and used in the following analysis. All unique sequences generated in this study were submitted to GenBank (accession numbers KF984250–KF984259; KF984275–KF984290; KF984473–KF984487: Table 4-3).

4.2.2. Data analyses

To visualize the relationships between haplotypes from two *Lycodes* species, statistically parsimony networks were created using TCS ver. 1.21 (Clement et al. 2000) with a connection limit of 90% probability. I used the program package Arlequin 3.5 (Excoffier and Lischer 2010) to estimate the genetic diversity of each species on the basis of three indices (*H*: number of haplotypes; *h*: haplotype diversity; π: nucleotide diversity). I estimated the past population growth by testing departure from selective neutrality by using Tajima's *D* (Tajima 1989) and Fu's *F*s. (Fu 1997) with 1,000 replicates. Mismatch distributions were also analyzed by calculating Harpending's raggedness index (HRI, Harpending 1994) to identify significant departures from model estimates assuming a sudden demographic expansion, with 1000 replicates. These tests were performed using Arlequin.

I used the Isolation with Migration program (IMa2, Hey 2010) to estimate the time since divergence (*T*) of the two *Lycodes* species. I used both COI and cyt*b* data in following analysis. All runs were performed in the 'MCMC mode' under the following conditions: –l (number of trees saved every 100 steps): 20,000 (i.e., 20,000,000 generations); –b (numbers of trees to be discarded as burn-in): 5,000,000 (i.e., 25%); –n (number of heated chains): 20; –fg (geometric heating scheme); and –g1: 0.99, –g2: 0.75 (heating terms of the geometric increment model). Prior values of θ (mutation-scaled effective population size), *t* (mutation-scaled age of divergence), and *m* (mutation-scaled pairwise migration rates) were determined after several trial runs. To convert mutation-scaled estimates of time since divergence (*t*) into age in calendar years (*T*), I used the mutation rate of cyt*b*, namely, 2.045% per My, for three-spined sticklebacks, *Gasterosteus aculeatus* (Mäkinen and Merilä 2008). and I tested whether applying both the two-fold higher and one-half lower rates would change my conclusions. The mutation rate for COI was estimated under the IMa model.

I visualized historical changes in effective population sizes using the Bayesian Skyline Plot (BSP: Drummond et al. 2005) implemented in the program package BEAST v1.8.0 (Drummond et al. 2012). I specified 30,000,000 generations with burn-in of 3,000,000 cycles (i.e., 10%) using both COI and cyt*b* sequence data. The mutation rate for cyt*b* was fixed at 2.045% per My as a prior value under the strict clock model, and the rate for COI was estimated under the lognormal relaxed clock model. I used an independent evolutionary model for each

locus with empirical base frequencies. The best-fitting evolutionary model for each data set was identified based on AIC, using the model test function implemented in MEGA 5. The results were checked using the program TRACER v1.5 (Rambaut and Drummond 2009) by testing that effective sample sizes for all demographic statistics were above 200 for each run.

4.3. Results

4.3.1. Population structure and genetic diversity

I determined partial sequences of mitochondrial COI (624 bp) and cyt*b* (756 bp) genes from 53 individuals of *L. japonicus* from the Sea of Japan and 37 *L. ocellatus* from the northwestern Pacific. Haplotype frequencies of each species and locus are given in Table 4-3. Haplotypes of *L. japonicus* and *L. ocellatus* were clearly segregated from each other, although they were connected by at least 14 steps in each mitochondrial locus (Fig. 4-3a and b). Average sequence divergence of *L. japonicus* and *L. ocellatus* was 2.9% in COI, which shows that the two species are closely related, considering the range of COI sequence divergence between sibling species of Chordata (1.2–12.9%: Hebert et al. 2003)

Lycodes ocellatus was characterized by higher genetic diversity than *L. japonicus*. I obtained more haplotypes from *L. ocellatus* than *L. japonicus*, and in addition, haplotypes of *L. ocellatus* were more divergent than those of *L. japonicus* (Table 4-4). Higher genetic diversity of *L. ocellatus* indicates a larger historical effective population size of *L. ocellatus* than *L. japonicus* (McCusker and Bentzen 2010).

4.3.2. Neutrality tests and mismatch distribution analysis

Both Fu's *F*s and Tajima's *D* were significantly different from zero in COI of both species and in cyt*b* of *L. ocellatus* (Table 4-4: $p < 0.05$) but were not significant for cyt*b* sequences of *L. japonicus*. The sudden expansion model was not rejected for all cases, as HRIs were not

significant (Fig. 4-4a, b), which implies that both species experienced sudden population growth.

4.3.3. Divergence age estimates based on the IMa model

The estimates for t (mutation rate-scaled time since divergence) and θ (mutation-scaled effective population size) for each *Lycodes* species were obtained under the IMa model. I converted θ into N_e/G (generation-scaled effective population size) and t into T (time since divergence in calendar years), by dividing each parameter by the mutation rate per sequence (Figs. 4-5, 4-6). If I assume the same generation time for both *Lycodes* species, the effective population size of *L. ocellatus* is twice as large as that of *L. japonicus* (Fig. 4-5). The estimated time since divergence of the two species fell into the mid- to late Pleistocene (0.2–0.8 Mya; Fig. 4-6). Even if the range of mutation rates is considered (1.0–4.0% per My), it is safe to presume that the two species diverged prior to the last glacial period (Fig. 4-6).

4.3.4. Historical changes in effective population size

BSP (Fig. 4-7) were constructed using the following evolutionary models for each partition: COI: HKY (Hasegawa et al. 1985) + Γ, cyt*b*: TN93 (Tamura and Nei 1993) + Γ. The time to the most recent common ancestor (TMRCA) of the extant haplotypes of *L. japonicus* was much more recent (~30,000 years ago) than that of *L. ocellatus*, which goes back to the early stage of the last glacial period (~80,000 years ago: Fig. 4-7). The effective population size of *L. ocellatus* has been consistently larger than that of *L. japonicus*. The population of *L. ocellatus* gradually increased during the last glacial period and ceased growing thereafter; meanwhile, the population size of *L. japonicus* is continuously increasing after the last glacial period.

4.4. Discussion

4.4.1. Divergence process of L. japonicus and L. ocellatus

The Sea of Japan is a semi-enclosed sea area connected to the Pacific Ocean, the East China Sea, and the Sea of Okhotsk by shallow straits (Tsugaru, Tsushima, Soya, and Mamiya Straits) which were suggested to have become land bridges or shallow seaways during the last glacial period (Ohshima 1990; Park et al. 2000). Previous studies have pointed out that sea-level changes induced divergence of deep-sea fish populations between the Sea of Japan and neighboring sea areas during the last glacial period (e.g. eelpout, *Bothrocara hollandi*: Kodama et al. 2008) and older glacial periods (snailfish, *Careproctus* spp.: Kai et al. 2011). However, each of these species is widely distributed in the northwestern Pacific Ocean, and it was not determined which strait isolated the populations.

The results of the population genetic analyses based on two protein-coding genes in mitochondrial DNA, viz. COI and cyt*b*, showed that *L. japonicus* and *L. ocellatus* are genetically distinct from each other, while still closely related (Fig. 4-3). The Tsugaru Strait, which currently separates the distribution ranges of the two species, may have played an important role in their speciation. The divergence age estimated under the IMa model suggests that these two species diverged during the mid-Pleistocene (0.2–0.8 Mya) when the climate changed drastically. The Tsugaru Strait (130 m depth) was 100 m became shallower than the present at least five times in the last one My, and at most may have become as shallow as fiver meters in depth 0.6 Mya (Fig. 4-6: estimated from Miller et al. 2005). *Lycodes* spp. are deep-sea fishes adapted to cold environments and in fact, *L. japonicus* and *L. ocellatus* have been recorded from 300–450 m depth in the Sea of Japan and 600 m in the northwestern Pacific, respectively (Hatooka 2013). Therefore, migration of adult *Lycodes* fishes through a shallow strait is unlikely. Larval dispersal is also impossible, because *Lycodes* spp. lack pelagic egg and planktonic larval phases (Matarese et al. 1989; Balanov et al. 2006). Although there is no geological evidence of the emergence of a Tsugaru land bridge, it is reasonable to assume allopatric divergence of the two species under the influences of mid-Pleistocene sea-level changes.

4.4.2. Demographic history of L. japonicus and L. ocellatus

The population of *L. ocellatus* in the northwestern Pacific is characterized by larger effective population size than *L. japonicus* in the Sea of Japan (Figs. 4-5 and 4-6). Relatively high genetic diversity of *L. ocellatus* also indicates that a large population size has been maintained in the northwestern Pacific under suitable conditions for *Lycodes* fishes (Table 4-4). Although the northwestern Pacific coast of Japan was influenced by seasonal sea ice coverage during the last glacial period (Harada et al. 2006; Harada et al. 2012), the bottom water did not become anoxic (Amano 2004). Population growth of coldwater *L. ocellatus* during the last glacial period may have been caused by the expansion of the cold deep-water zones in the northwestern Pacific.

BSP strongly suggested that population expansion of *Lycodes* species occurred during the past 60,000 years (Fig. 4-7). While Fu's F_S and Tajima's *D* were not significant for cyt*b* of *L. japonicus*, HRI did not reject population expansion. Ramos-Onsins and Rozas (2002) reported that the statistical power of HRI, Fu's F_S, and Tajima's *D* tests drastically change depending on the sample size, the number of segregating sites, the degree of expansion, and time since the expansion.

The population of *L. japonicus* has expanded recently, and such a rapid population growth after the glacial period is a common phenomenon among deep-sea species of the Sea of Japan (Shirai et al. 2006; Adachi et al. 2009). Strong density stratification of the water column developed during the LGM, which resulted in fatally anoxic sea-bottom conditions in most of the Sea of Japan (Itaki et al. 2004). The present samples of *L. japonicus* showed a younger coalescent age than *L. ocellatus*, which may suggest that the modern population of *L. japonicus* was established by a few ancestors, which survived the last glacial period in a glacial refugium in the Sea of Japan. While the location of the refugium cannot be discussed based on my present results, previous population genetic (Kojima et al. 2000) and geological (Gorbarenko and Southon 2000) studies have inferred the area that was favorable for deep-sea organisms in the Sea of Japan even during the LGM.

4.4.3. Climatic oscillation-induced diversification of deep-sea fishes in the northwestern Pacific marginal seas

Some deep-sea fishes are widely distributed and have a circumglobal distribution (Varela et al. 2012), and each species tends to be genetically homogeneous over its distribution range (Adachi et al. 2009; White et al. 2011; Takeshima et al. 2011). In the case of allopatric divergence of sibling species, sometimes it is difficult to attribute vicariance to a specific geological event because their present distribution ranges largely overlap (Stefanni and Knutsen 2007). My results show that a pair of sibling species of deep-sea demersal fishes, *L. japonicus* and *L. ocellatus*, diverged during the mid-Pleistocene. As the distribution ranges of these two species are limited to areas separated only by the shallow Tsugaru Strait, their divergence is attributable to sea-level changes during the mid-Pleistocene. Furthermore, my data infer population reduction in the Sea of Japan during the last glacial period due to the unfavorable environmental conditions that may have restricted connectivity among local populations, which might have accelerated genetic divergence from the northwestern Pacific population. Previous biogeographic studies have pointed out that climatic oscillation induced the allopatric divergence of marine species in the northwestern Pacific marginal seas (Nishimura 1968; Briggs 1974). Molecular phylogenetic evidence has also suggested the importance of land bridge formation and fractionation of marine habitats during the mid-Pleistocene glacial periods for the establishment of present marine biodiversity (Yamazaki et al. 2013). My data present additional evidence of such glacial-induced allopatric divergence between the Sea of Japan and the adjacent sea areas. Future population genetic studies in the northwestern Pacific will provide a new timeframe for these diversification events, and may reveal the modes of speciation of deep-sea organisms including the allopatric processes described here.

Tables

Table 4-1 Morphological variations of *Lycodes* spp. distributed in the Sea of Japan and adjacent sea areas (modified from Toyoshima, 1985). **Bold** type indicates species of which samples were used in this chapter.

Species	Dorsal fin rays	Anal fin rays	Vertebrae		Body coloration
			Abdominal	Caudal	
L. japonicus	**79-84**	**69-73**	**19-20**	**68-72**	**Brown, with irregular blotches**
L. ocellatus	**81-89**	**69-76**	**19-20**	**75-78**	**Dark brown, with irregular blotches**
L. hubbsi	104-109	89-94	20-21	89-94	Black, with vertical lines
L. matsubarai	93-99	77-84	21-22	81-85	Pale brown, with vertical lines
L. sadoensis	72-78	62-65	20	62-67	Pale brown, with irregular vertical lines
L. tanakae	96-98	77-79	25-26	77-81	Brown, with vertical lines in young/various blotches in adult
L. toyamensis	90-100	75-84	21-23	76-85	Black

Table 4-2 Sampling date, locations and the number of the samples of *Lycodes* species used in this study. The name of vessels and their affiliation were also provided.

Samples	Date	Latitude	Longitude	Depth (m)	*n*	Vessels
L. japonicus						
J-1	Jun 2011	36˚15.66 N	135˚29.32 E	450	8	T/S Tanshu-Maru, Hyogo pref.
J-2	Jun 2012	36˚07.01 N	135˚37.43 E	347	45	T/S Tanshu-Maru, Hyogo pref.
total					53	
L. ocellatus						
O-1	Oct 2009	38˚22.62 N	142˚10.60 E	663	21	Wakataka-Maru, TNFRI*
O-2	Oct 2009	38˚22.18 N	142˚13.88 E	755	12	Wakataka-Maru, TNFRI*
O-3	Jul 2012	36˚59.27 N	141˚38.64 E	561	4	Tansei-Maru, University of Tokyo
total					37	
total					**90**	

* Tohoku National Fisheries Research Institute, Fisheries Research Agency

Table 4-3 Nucleotide variation and frequencies of mitochondrial DNA haplotypes of cytochrome *c* oxidase subunit I (COI) and cytochrome *b* (cyt*b*) genes: (a) COI of *L. japonicus*; (b) COI of *L. ocellatus*; (c) cyt*b* of *L. japonicus*; (d) cyt*b* of *L. japonicus*

(a)

Haplotype	GenBank Accession numbers	Nucleotide position from 5' end								*n*
		45	163	183	270	401	432	504	540	
CO01	KF984275	C	C	A	G	C	G	C	C	40
CO02	KF984276	.	.	.	.	.	.	T		4
CO03	KF984277	.	.	.	.	.	.	.	T	4
CO04	KF984278	.	.	G	.	.	.	.	.	1
CO05	KF984279	T	.	.	.	T	.	.	.	1
CO06	KF984280	.	T	G	.	.	.	.	.	1
CO07	KF984281	.	.	.	.	.	T	.	.	1
CO08	KF984282	.	.	.	A	.	.	.	T	1
total										53

(b)

Haplotype	GenBank Accession numbers	Nucleotide position from 5' end										*n*
		69	150	288	325	369	401	435	442	549	573	
CO09	KF984250	T	C	G	T	C	C	G	G	G	T	4
CO10	KF984251	C	.	.	.	.	.	.	.	.	.	23
CO11	KF984252	.	.	.	.	.	.	A	.	.	.	3
CO12	KF984253	C	.	.	C	.	.	.	.	.	.	1
CO13	KF984254	.	.	.	.	.	.	.	A	.	.	1
CO14	KF984255	C	.	.	.	.	.	.	.	A	.	1
CO15	KF984256	C	.	.	.	.	T	.	.	.	.	1
CO16	KF984257	C	.	A	.	A	.	.	.	.	.	1
CO17	KF984258	C	T	.	.	.	.	.	.	.	.	1
CO18	KF984259	C	.	.	.	.	.	.	.	.	C	1
total												37

(c)

Haplotype	GenBank Accession numbers	Nucleotide position from 5' end						*n*
		173	231	303	474	582	708	
Cb01	KF984283	G	C	G	C	G	C	27
Cb02	KF984284	.	.	A	.	.	T	3
Cb03	KF984285	.	.	.	.	A	T	7
Cb04	KF984286	.	.	.	.	.	T	11
Cb05	KF984287	A	.	.	.	.	T	2
Cb06	KF984288	.	.	A	.	.	.	1
Cb07	KF984289	.	.	.	T	.	.	1
Cb08	KF984290	.	T	.	.	.	T	1
total								53

(d)

Haplotype	GenBank Accession numbers	Nucleotide position from 5' end																				*n*
		57	117	129	151	173	239	261	300	303	346	360	432	459	519	525	609	687	711	732	747	
Cb09	KF984473	G	G	T	G	G	T	G	T	G	A	G	A	A	T	T	C	C	C	T	A	4
Cb10	KF984474	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	13
Cb11	KF984475	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	1
Cb12	KF984476	.	.	.	.	.	.	.	.	A	.	.	G	.	C	.	.	.	.	.	.	3
Cb13	KF984477	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	4
Cb14	KF984478	A	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	1
Cb15	KF984479	A	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Cb16	KF984480	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	3
Cb17	KF984481	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	1
Cb18	KF984482	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	1
Cb19	KF984483	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	1
Cb20	KF984484	A	A	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	1
Cb21	KF984485	A	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.	.	.	.	1
Cb22	KF984486	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	1
Cb23	KF984487	A	.	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.	T	.	.	1
total																						37

Table 4-3 Number of individuals (*N*), number of haplotypes (*H*), haplotype diversity (*h* ± SD), nucleotide diversity (π ± SD), Fu's F_S, and Tajima's *D* indices of neutrality were shown for *Lycodes japonicus* and *L. ocellatus*. Significant P values (P<0.05) are indicated by asterisks.

Species	Locus	*N*	*H*	*h*	π	Fu's F_S	*Tajima*'s *D*
L. japonicus	COI	53	8	0.425 ± 0.083	0.0009 ± 0.0008	–5.41*	–1.81*
	Cyt*b*	53	8	0.688 ± 0.054	0.0014 ± 0.0001	–0.56	–2.79
L. ocellatus	COI	37	10	0.607 ± 0.090	0.0015 ± 0.0012	–6.65*	–1.84*
	Cyt*b*	37	15	0.856 ± 0.047	0.0028 ± 0.0002	–8.08*	–1.85*

Figures

Fig. 4-1 *Lycodes japonicus* collected from Wakasa Bay in the Sea of Japan (a) and *L. ocellatus* collected from the northwestern Pacific Ocean (b).

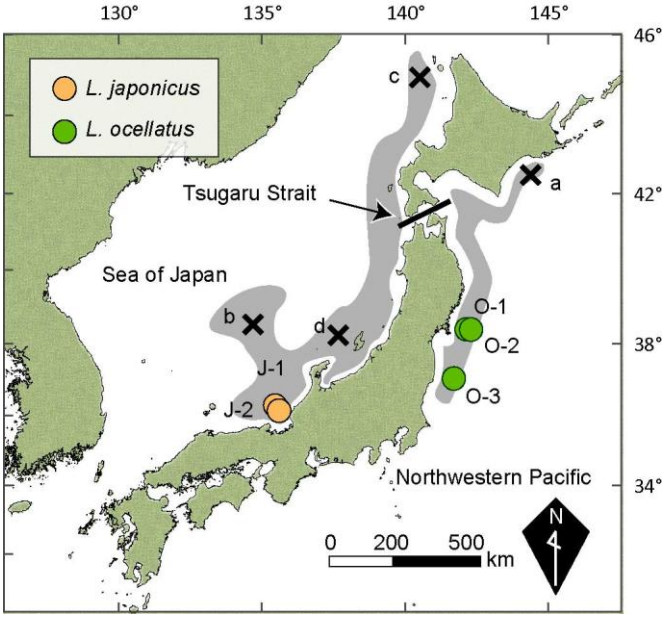

Fig. 4-2 Study area, showing sampling locations (circles). Shaded areas show the geographic distribution of *Lycodes japonicus* and *L. ocellatus* (Toyoshima 1985; Hatooka 2013). Xs show the sites where *Lycodes* fishes were recorded (a, Nakabo et al. 2013; b, Okiyama 2004) or museum collections were deposited (c, Kyoto University: FAKU200482–FAKU200484; d, National Museum of Nature and Science: NSMT-P 111910).

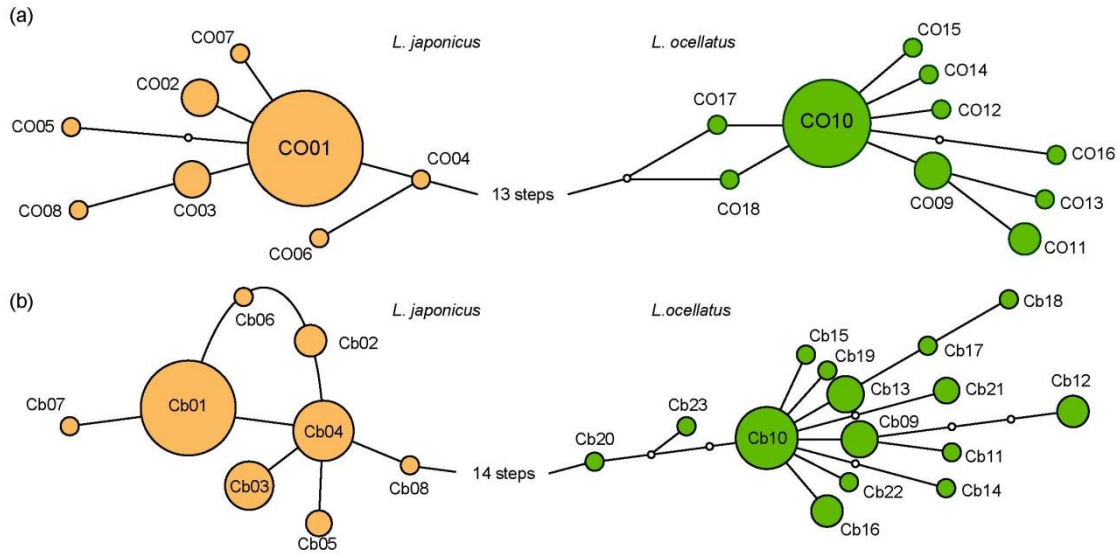

Fig. 4-3 Statistically parsimony networks of haplotypes from *Lycodes japonicus* and *L. ocellatus* based on nucleotide sequences of cytochrome *c* oxidase I (COI: a) and cytochrome *b* (cyt*b*: b) genes. Circle size is proportional to the number of individuals of each haplotype, and labels indicate the names of haplotypes. Small open circles indicate intermediate haplotypes that were not sampled.

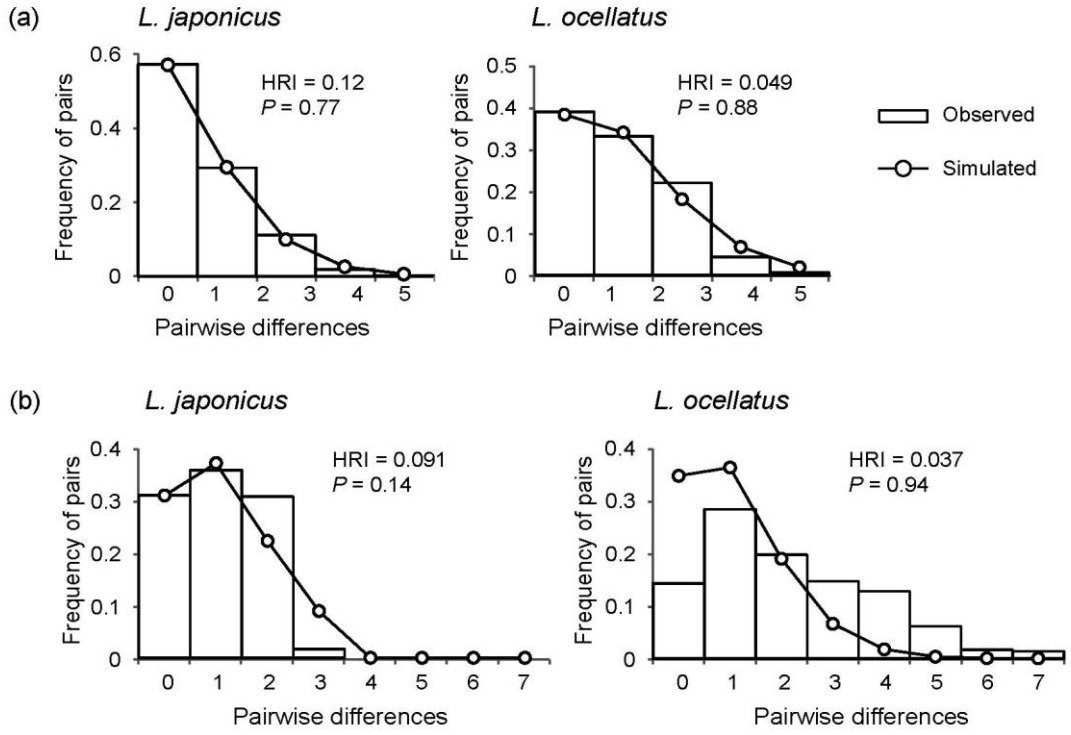

Fig. 4-4 Mismatch distributions based on cytochrome *c* oxidase I (COI: a) and cytochrome *b* (cyt*b*: b) sequences of *Lycodes japonicus* and *L. ocellatus*. Lines represent the expected frequencies of mismatches under the sudden expansion model. Harpending's raggedness index (HRI) values of observed mismatch distributions and corresponding *p*-values are also shown.

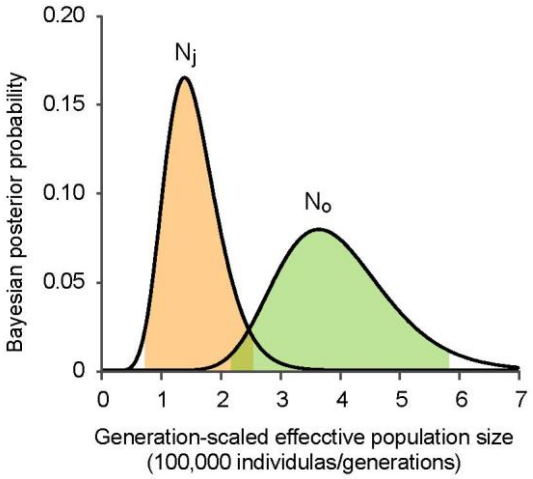

Fig. 4-5 Posterior distribution of generation-scaled effective population size of *Lycodes japonicus* (Nj) and *L. ocellatus* (No), estimated from the Isolation with Migration model using multi-locus data from cytochrome *c* oxidase I (COI) and cytochrome *b* (cyt*b*) sequences.

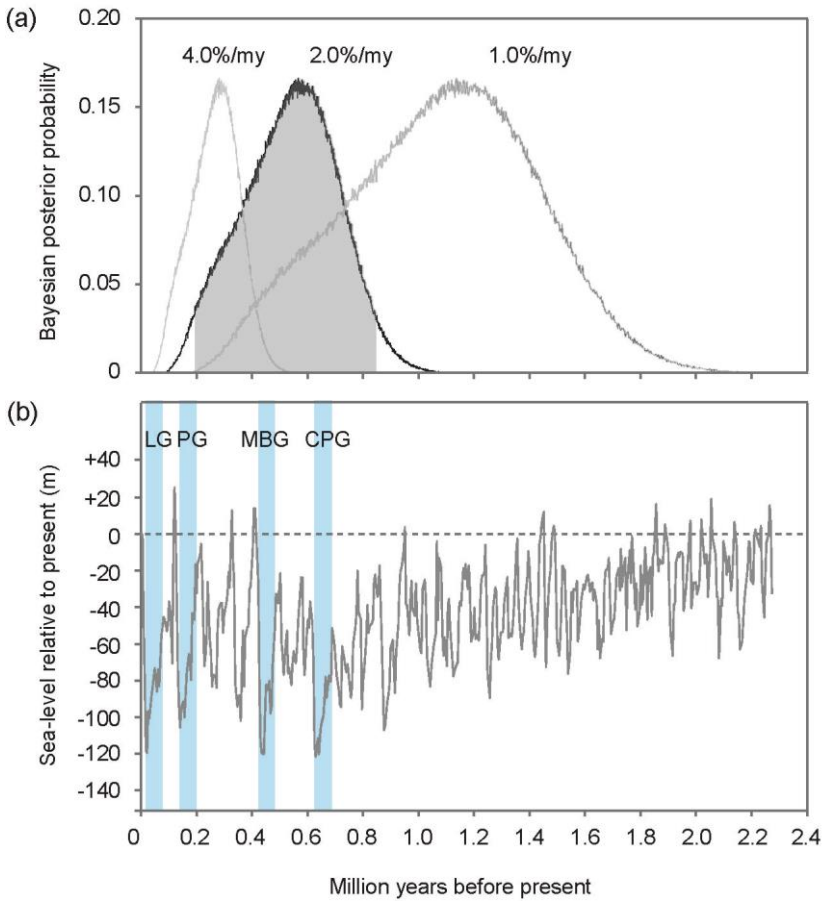

Fig. 4-6 (a) Posterior distribution of time since divergence (*T*) under the Isolation with Migration model and the range of mutation rate (1.0–4.0% per million years: My in cytochrome *b*). Shaded areas show 95% highest posterior density intervals. (b) Global trends of sea-level change during the last two My (modified from Miller et al. 2005). Dashed line indicates the present sea-level. Blue areas indicate past glacial periods. Transitions between glacial and interglacial conditions follow the recommendations of Ruzzante et al. (2008). Abbreviated climatic events include the Last Glaciation (LG), the Penultimate Glaciation (PG), the Mid-Brunhes Glaciation (MBG), and the Coldest Pleistocene Glaciation (CPG).

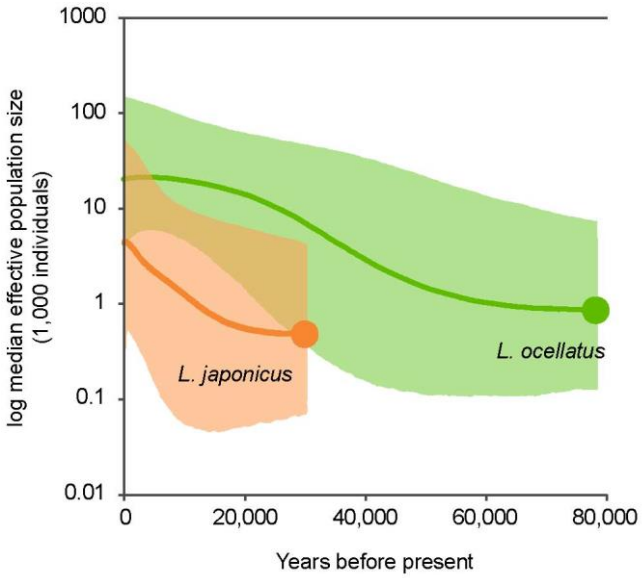

Fig. 4-7 Bayesian skyline plots illustrating demographic changes in the populations of *Lycodes japonicus* and *L. ocellatus*. Solid circles show times to the most recent common ancestor of sampled haplotypes. Lines indicate the median estimates and shaded areas show 95% highest posterior density intervals.

General discussion

The evolutionary process of the deep-sea fishes in the subfamily Lycodinae was discussed throughout this thesis, in terms of the impacts of drastic climate changes of the Quaternary ice ages. The molecular phylogenetic evidences showed the relationships among the taxa in the subfamily Lycodinae and inferred that the adaptation to the deep-sea bottom environment had independently advanced in the three lineages in the subfamily. I also found several pairs of sibling species in the subfamily Lycodinae, which was assumed to have been diversified under strong influences of the Quaternary climatic oscillation (Chapter 1). The present geographic distribution of six Lycodinae species was compared in the Chapter 2. The spatial distribution of some Lycodinae species was restricted within the specific regions in the southwestern Sea of Japan. When the connectivity between such regions was lost, allopatric divergence may have occurred. Such an allopatric mechanism was shown to have contributed to the formation of the population structure of *L. matsubarai*. The populations in the Sea of Japan and the Sea of Okhotsk were suggested to have been diverged from each other, due to the low sea-level stand during the last glacial period (Chapter 3). The speciation of *L. japonicus* and *L. ocellatus* was also suggested to have been driven by the geological isolation during the mid-Pleistocene (Chapter 4). In addition, the results of the Chapters 4 and 5 showed the contrasting population histories in the Sea of Japan and the adjacent seas.

The glacial impacts on the marine organisms in the northwestern Pacific seas

It is evident that the marine taxa in the Sea of Japan were strongly influenced by the serious environmental changes during the last glacial period but they passed through different processes in the shallow and deep waters. Kojima et al. (2004) showed the large reduction of population size during the LGM and subsequent population expansion for the intertidal snail *Batillaria cumingi* in the Sea of Japan, owing to the cold and low-salinity surface water caused by the inflow of a significant amount of freshwater into the surface layer. Kokita and Nohara (2011) and Hirase et al. (2012) also found remarkably similar phenomenon in the ice goby

Leucopsarion petersii and the forktongue goby *Chaenogobius annularis* in the Sea of Japan, respectively. Deep-sea species have also been shown to have experienced such a genetic bottleneck and subsequent population expansion (sailfin sandfish, *Arctoscopus japonicus*, Shirai et al. 2006; Japan Sea eelpout, *Bothrocara hollandi*, Kodama et al. 2008; darkfin sculpins, *Malacocottus* spp., Adachi et al. 2009). These phenomena were, however, attributed to the anoxic condition in the bottom layer. Previous palaeoceanographic studies suggested that the strong density stratification developed within the water column during the LGM, which resulted in fatally anoxic sea-bottom conditions in most deep-sea parts of the Sea of Japan (Itaki et al. 2004). The glacial population bottleneck of the *L. matsubarai* and *L. japonicus* shown in this study are thus concordant with the previous knowledge of the deep-sea species, which shows that various marine organisms in the Sea of Japan were affected by the severe environmental changes in the Sea of Japan during the last glacial period.

The present results are indicative of glacial refugia in the Sea of Japan. The present-day distribution range of *L. matsubarai* is restricted within the westernmost area of the Sea of Japan (Fig. 3-4). This species was suggested to have survived in the Sea of Japan during the last glacial period as the divergence between the Sea of Japan and the Sea of Okhotsk populations predates the post-glacial population expansion in the Sea of Japan (Fig. 4-7). I therefore assume that the westernmost area in the Sea of Japan played an important role as a refugium during the last glacial period. The palaeoceanographic studies also support this assumption. A fraction of the palaeo-Tsushima Current continually flowed into the Sea of Japan presumably through the Tsushima Strait, even during the LGM (Park et al. 2000). Moreover, Gorbarenko and Southon (2000) suggested that the southwestern shelf area in the Sea of Japan near the Tsushima Strait did not become anoxic throughout the last glacial period. The population history and the present-day distribution of *L. japonicus* also infer the refugium in the middle part of the Sea of Japan, namely, Wakasa Bay. To date, no palaeoceanographic study have been carried out in this sea area and there was possibly another refugium in the Sea of Japan during the last glacial period.

The demographic responses of the deep-sea Lycodinae species to the glacial environmental changes in the Sea of Okhotsk and the northwestern Pacific Ocean were striking contrast to those in the Sea of Japan. Abundance of *L. matsubarai* in the Sea of Okhotsk

gradually increased even during the LGM and suddenly decreased recently (Figs. 3-6, 3-7). *L. ocellatus* in the northwestern Pacific Ocean experienced population growth during the LGM (Fig. 4-7). Kodama et al. (2008) suggested that the Japan Sea eelpout have not experienced the sudden population expansion in the Sea of Okhotsk. The shallow-water taxa in the Pacific coast of the Japan Archipelago were also suggested to have maintained relatively stable population even during the last glacial period (Kokita and Nohara 2011; Hirase et al. 2012). These results suggest that the glacial responses in these areas were common among some shallow and deep-sea fishes in these sea areas.

The responses to the recovery from glacial environmental changes of marine species are, generally, characterized by the sudden population expansion. Previous studies upon the coastal marine fishes have suggested that the recent population expansion is common in these species and were likely triggered by the drastic climate changes during the Quaternary (Avise 2000). More recently, Varela et al. (2012) showed that the effective population size of the orange roughy, *Hoplostethus atlanticus*, dramatically increased during the interglacial periods. They inferred that the marine environmental changes associated with the glacial and interglacial events in the Quaternary had great effects in the demographic history of this cosmopolitan deep-sea species. Although the ages of the demographic expansion have not been determined, genetic bottlenecks were also reported for the alfonso *Beryx decadactylus* (Friess and Sedberry 2011) and the black scabbardfish *Aphanopus carbo* (Stefanni and Knutsen 2007). The population expansion related to the glacial environmental changes was regarded as a common phenomenon in most marine taxa.

Population fragmentation in the glacial period and subsequent range expansion have been repeated in terrestrial taxa during the Quaternary ice ages, which have contributed to their speciation and the diversification (Hewitt 1996; Provan and Bennett 2008). In this study, I therefore hypothesized that such a mechanism also drove the diversification of marine organisms in the northwestern Pacific area. As the environmental condition was more favorable during the interglacial than the glacial periods, geographic barriers which had isolated the local populations were lost in the former periods, and subsequently, re-colonization from the glacial refugia would be induced.

Evolutionary process of the deep-sea fishes in the subfamily Lycodinae

The species in the subfamily Lycodinae were inferred to have diverged under the strong influences of the environmental changes during the Quaternary ice ages in this study. I showed that the divergence between three lineages of the subfamily Lycodinae most probably occurred during the Pleistocene (Fig. 1-5) in the Chapter 1. In the Chapter 4, I also showed that the two Lycodinae species, *L. japonicus* and *L. ocellatus*, diverged during the mid- to late-Pleistocene (Fig. 4-6), likely due to formation of the Tsugaru land bridge by the lowered sea-level. Briggs (1974) previously hypothesized that the Quaternary glacial cycles induced isolation of the Seas of Japan and Okhotsk, which permitted the evolution of many endemic species. More recently, Kai et al. (2011) showed that the divergence between the lineages of a snailfish, *Careproctus rastrinus*, which is thought to reflect the geographic isolation of the populations. The scenario assuming that the geographic isolation among the marginal seas of northwestern Pacific Ocean acted as a driver of the speciation would be applicable to the subfamily Lycodinae.

The parallel acquisition of the cartilaginous submental crest during the Pleistocene was inferred in the Chapter 1, which led several lineages of Lycodinae to diversify above the deep-sea floor (Fig. 1-5). Nishimura (1983) pointed out that the decrease of water temperatures during the late Pliocene allowed the cold-water species' divergence and dispersal in the North Pacific. The range expansion of the cold-water Lycodinae species might have been induced by such changes in the global climates, in combination with the evolutionary adaptation.

Future perspectives

I reconstructed the population histories of *L. matsubarai* in the Chapter 3 and *L. japonicus* and *L. ocellatus* in the Chapters 4, respectively. They showed a consistent pattern depicting the responses of the deep-sea species to the drastic environmental changes during the last glacial period in the seas of northwestern Pacific. To address the general trends in the glacial demographic responses, however, further investigations on the deep-sea organisms are required

to compare the population history among deep-sea fishes with various ecological/biological traits. In addition, extensive sampling over the geographic range of each target species would be necessary to reveal detailed population history. My samples did not fully cover the entire ranges of the target species in the Chapters 3 and 4. It is thus possible that the sampling bias would have influenced my conclusion. Exhaustive sampling that fully covers their ranges is therefore desired to confirm the conclusions of this study.

I examined 23 taxa in the large family Zoarcidae and showed the credible phylogenetic relationships among them in the Chapter 1. However, this family encompassing more than 260 species in the coastal areas to abyssal zones worldwide, and to solve the taxonomic problems which raised in this thesis and to reconstruct clearer phylogenetic framework, therefore, comprehensive taxon sampling is indispensable. With the additional specimens of taxa which were not included in this study may bring new ideas about the evolutionary in this deep-sea fish group. In addition, more robust timeframe for the diversification process of the subfamily Lycodinae is required to elucidate the entire picture of the evolutionary history. Unfortunately, no fossil record of zoarcid species is available and thus the age of divergence between zoarcid taxa should have been deduced using the molecular clock assumption in this study. A possible solution is the usage of the external calibration points at multiple nodes of the sister taxon of the family Zoarcidae, which frequently occurs in the fossil i.e., Gasterosteidae (sticklebacks), Sebastidae (rockfishes) and Cottidae (sculpins). When the age of divergence is estimated based on more credible information, the questions arose in this thesis, for example, whether the diversification occurred synchronously and whether the allopatric mode of speciation has been prevalent in this family, would be fully answered.

Acknowledgements

In the first place, I would like to express my sincere gratitude to Professor Dr. Shigeaki Kojima of the Department of Science, the University of Tokyo, who gave me the opportunity to carry out this study and detailed advice for this study.

I am also deeply grateful to Dr. Hirohiko Takeshima for his guidance and encouragement throughout this study. I could have not achieved this study without his kind and constructive feedback.

This study would not have been possible without the assistance and contribution of a number of persons. My special thanks go to Drs. Yuji Ueda and Kunihiro Fujiwara (Japan Sea National Fisheries Research Institute, Fisheries Research Agency) who provided me valuable specimens from the Sea of Japan and also many insightful suggestions upon my manuscript, Drs. Masaki Itou, Tsutomu Hattori and Yoji Narimatsu (Tohoku National Fisheries Research Institute, Fisheries Research Agency) who kindly helped with the sampling off Fukushima, Drs. Masayuki Chimura, Tomonori Hamatsu and Hiroshige Tanaka (Hokkaido National Fisheries Research Institute, Fisheries Research Agency) who provided me specimens around Hokkaido, Drs. Yoshiaki Kai (Kyoto University), Nozomu Muto (The Research Institute for Humanity and Nature) and Mr. Akira Toukairin (Kyoto University) who helped in the collecting specimens onboard.

I am indebted to all the staffs and crews of the training ship *Tanshu Maru*, research vessels *Wakataka Maru*, *Daigo Kaiyo Maru*, *Tansei Maru* and *Hokko Maru*, and supporting staffs of Fisheries Research Agency, Nippon Kaiyo Ltd., Atmosphere and Ocean Research Institute (AORI) and Marine Works Japan Ltd.

I am also appreciate all the staffs and the members in the laboratory of the Benthos section of AORI for their technical supports, valuable comments, constructive suggestions and warm encouragement during this study. I would like to thank my friends for the encouragement.

Finally, I would like to thank my parents for tremendous supports and sincere encouragements. This study would not have been possible without their help.

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Appendix

Unambiguously aligned partial nucleotide sequences of the mitochondrial cytochrome *b* (cyt*b*), cytochrome *c* oxidase subunit I (COI), 12S rRNA (12S) and 16S rRNA (16S) genes, and nuclear Rhodopsin retrogene (Rhodopsin) from 23 Zoarcid species and *Stichaeus nozawai*, used for molecular phylogenetic analyses in the Chapter 2. Insertions and deletions of specific nucleotides indicated by dashes (–).

Cytochrome *b* (*cytb*)

```
Bothrocara hollandi           A A A A T C G C A A A T A G C G C A C T A G T T G A C C T A C C C G C C C C C T C C A A T A T C T C A G T G T G G T G A [ 60]
Davidijordania poecillimon    . . . . . . . . . . . . . . . . . . . . C . . G . . . . . . . G . . . . . . . T . . . . . C . . . . . . . . A . . . . . . . [ 60]
Krusensterniella maculata     . . . . . . . . . . . . . . . A T . . C . . . . . G . . . . . C . . . . . . . . . . . . C . . . . . . . . . . . . . . . [ 60]
Lycenchelys albomaculata      . . . . . . . . . . . C . A . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycenchelys aurantiaca        . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycenchelys melanostomias     . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodapus  microchir          . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes albonotatus           . . . . . . . . . . . . . . . A . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes hubbsi                . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes japonicus             . . . . . . . . . . . . . . . A . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes matsubarai            . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes nakamurai             . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes ocellatus             . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes pectoralis            . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . T T . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes raridens              . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes sadoensis             . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes soldatovi             . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes tanakae               . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes teraoi                . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes toyamaensis           . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Stichaeus nozawai             . . . . . T . . . . . C . A T . . . . . G . . . . . . . C . . A . . . . . . . T . . C . . . . . . . A . . A . . . [ 60]
Zoarces americanus            . . . . . T . . . . . . A T . . . . . . . . . . . . . . C . . . . . . . . . . . C . . T . . . . . A . . A . . . [ 60]
Zoarces elongatus             . . . . . T . . . . . . A T . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . A . . . [ 60]
Zoarces gilli                 . . . . . T . T . . . . . A T . . . . . . . . . . . . . . C . . A . . . . . . . . . . . T . . G . . A . . A . . . [ 60]

Bothrocara hollandi           A A C T T T G G C T C C C T G C T C G G C C T T T G C T T A A T T A T T C A A A T C C T T A C C G G G A C T C T T C C T C [120]
Davidijordania poecillimon    . . . . . . . . T . . . . . . . . . T . . . . . C . . . . . . G . . . G . C . . . . . . . . . . . . . A . . . . . T . . . . . [120]
Krusensterniella maculata     . . . . . . . . T . . . . . A . . T . . . . . . . . . . . . G . . . G . C . . . . . . . . . . . . . A . . . . . T . . . . . G [120]
Lycenchelys albomaculata      . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . G . . . . . . . [120]
Lycenchelys aurantiaca        . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycenchelys melanostomias     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [120]
Lycodapus  microchir          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes albonotatus           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A [120]
Lycodes hubbsi                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes japonicus             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes matsubarai            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes nakamurai             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes ocellatus             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes pectoralis            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes raridens              . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes sadoensis             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes soldatovi             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes tanakae               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes teraoi                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes toyamaensis           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Stichaeus nozawai             . . . . . . . . . . . . . . T . A . . T . . . . . . . . . . G G . A . . C . . . . . T . . . . . A . . . . . . . . T . . . [120]
Zoarces americanus            . . . . . . . . . T . . . . . T . . A . . . . . . . . . . . G . . . . . . . . . . . . . . C . . A . . . . . T . . . . . . [120]
Zoarces elongatus             . . T . . . . . . . . . . . . A . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . A . . . . . T . . . . . . [120]
Zoarces gilli                 . . . . . . . . . T . . . . . C . . G A . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . T . . T . . . . . . [120]

Bothrocara hollandi           G C T A T A C A T T A C A C C C C G G A T A T C G C A A C C G C C T T C T C C T C T A T C G G C C A C A T T T G T C G A [180]
Davidijordania poecillimon    . . . . . . . . . C . . . . . T T . C . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . . . . . . . C . . C . . . [180]
Krusensterniella maculata     . . . . . . . . . C . . . . . . . T . T . . . . . . . . . . . . T . . . . . . . . . . . . T . . T . . . . . . G C C . . . [180]
Lycenchelys albomaculata      . . C . . . . . . . . . . . . . . T . . C . . . . . . . . . . . T . . . . . . . . . . . . T . . T . . . . . . . C . . . [180]
Lycenchelys aurantiaca        . . . . . . . . . C . . . . . . G . T . . C . . . . . . . . . . T . . . . . . . . . . . . T . . T . . . . . . . C . . . [180]
Lycenchelys melanostomias     . . . . . . . . . C . . T . . . . . C . . . . . . . A . . . . . . . . . . . . T . . . . . . . . . . . . C . . . . . . . [180]
Lycodapus  microchir          . . . . . . . . . . . . T . . . . . C . . C . . . . . . . . . . T . . . . . . . . . . . G . T . . . . . T . . . . . . . [180]
Lycodes albonotatus           . . C . . G . . C . . . . . . . . . C . . C . . . . . . . . . . T . . . . . . . . . . . G . T . . G . . . . . . . . . . [180]
Lycodes hubbsi                . . . . . . . . . C . . T G . . . . C . . C . . . . . . . . . . T . . . . . . . . . . . . T . . T . . . . . C . . . . . [180]
Lycodes japonicus             . . C . . . . . . . . . T . . . . . C . . C . . . . . . . . . . T . . . . . . . . . . . . T . . T . . . . . . . . . . . [180]
Lycodes matsubarai            . . C . . . . . . . . . . . . . T . C . . . . . . . . . . . . T . . . . . . . . . . T . . . . . . . . . . C . . . [180]
Lycodes nakamurai             . . . . . . . . . . . . . . . T . C . . . . . . . . . . . . T . . . . . . . . . . C . . . . . . . . . . . . C . . . [180]
Lycodes ocellatus             . . . . . . . . . C . . T G . . . . C . . C . . . . . . . . . T . . . . . . . . . G . T . . T . . . . . . . . . . . [180]
Lycodes pectoralis            . . . . . . . . . . . . . . . T . C . . . . . . . . . . . . T . . . . . . . . . T . . C . . . . . . . . . . . . C . . . [180]
Lycodes raridens              . . . . . . . . . C . . . . . . T . T . . . . . . . . . . . T . . . . . . . . . T . . G . T . . T . . . . . . . . C . . . [180]
Lycodes sadoensis             . . . . . . . . . C . . T . . . T . C . . C . . . . . . . . T . . . . . . . . . G . A . . T . . . . . . . . . . . . . [180]
Lycodes soldatovi             . . C . . . . . C . . . . . . . . C . . . . . . . . . . . T . . . . . . . . . T . . . . . . . . . . . . . . C . . . [180]
Lycodes tanakae               . . . . . . . . . T . . . G . T . . . . . . . . . . . . . T . . . . . . . . . T . . . G . T . . . . . . . . . C . . . [180]
Lycodes teraoi                . . C . . . . . . T . . . T . C . . C . . . . . . . . . T . . . . . . . . . G . G . . T . . . . . . . . . . . . . [180]
Lycodes toyamaensis           . . . . . . . . . T . . . T . C . . C . . . . . . . . . T . . . . . . . . . G . T . . G . . T . . . . . . . . . . . [180]
Stichaeus nozawai             . . C . . . . . . . . . . . T . T . . C . . . . . . . . . T . . . . . . . . . T . . . G . T . . T . . . . . . . . . . [180]
Zoarces americanus            . . C . . . . . . C . . T . . . T . C . . C . . . . . . . . T . . . . . . . . . T . . . G . T . . T . . . . . . . . C . . . [180]
Zoarces elongatus             . . C . . . . . . C . . . . . . . T . C . . C . . . . . . . . T . . . . . . . . . . . . G . T . . T . . . . . . . . . . . [180]
Zoarces gilli                 . . C . . . . . . C . . . . . . . T . T . . . . . . . T . . . . . . . . . . . . . . . . G . A . . . . . . . . . . . . . [180]

Bothrocara hollandi           G A C G T C A A C T A C G G C T G G C T C A T C C G C A A C C T C C A C G C C A A C G G C G C C T C C T T T C T T C T T C [240]
Davidijordania poecillimon    . . . . . . . . . . . . . . . . . . . . . . . . . . A . . T . . . . . . . . . . . . . . . . . . T . . A . . . . . . . . . A . . . . . . . . T . . . [240]
Krusensterniella maculata     . . . . . . . . . . . . . . . . . . . . . . . . . . A . . A . . . . . . . . . . . . . . . . . . T . . T . . . . . . . . . A . . T . . . . . T . . . [240]
Lycenchelys albomaculata      . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycenchelys aurantiaca        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycenchelys melanostomias     . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodapus  microchir          . . . . . T . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes albonotatus           . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . [240]
Lycodes hubbsi                . . . . . T . . . . . T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes japonicus             . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . T . . . . . G . G . . . . . . . . . . [240]
Lycodes matsubarai            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes nakamurai             . . . . . . . . . . . . . . . . . . . . . . . . . . A T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes ocellatus             . . . . . T . . . . . T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . [240]
Lycodes pectoralis            . . . . . . . . . . . . . . . . . . . . . . . . . . A T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes raridens              . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes sadoensis             . . . . . T . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . [240]
Lycodes soldatovi             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes tanakae               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes teraoi                . . . . . T . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . [240]
Lycodes toyamaensis           . . . . . T . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . [240]
Stichaeus nozawai             . . . . . . . . . . . . . . . . . . . . . . A . . T . . . . . . A . . . . . T . . T . . . . . . . . . . . A . . T . . . . . T . . T [240]
Zoarces americanus            . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . [240]
Zoarces elongatus             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . A . . . . . . . . . . [240]
Zoarces gilli                 . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . [240]
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Cytochrome *b* (*cytb*)

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Bothrocara hollandi                A T C T G C A T T T A C A T G C A C A T C G G C C G C G G G C T G T A T T A C G G C T C C T A T C T C T A T A A A G A A [300]
Davidijordania poecillimon         . . . . . T G . C . . . C . A . . . . . . . . . . . . T . . A . . A . . C . . . . . . . . . . . . C . . . . . C . . G . . G [300]
Krusensterniella maculata          . . . . . C . . T . . . . . . . . . . . . . . T . . A . . A . . C . . . . . . . . . . . . C . . . . . C . . G . . . [300]
Lycenchelys albomaculata           . . . . . T . . . . . A T . A . . . . . T . . . . . T . . . . . [300]
Lycenchelys aurantiaca             . . . . . C . . T . . . . . G . . . . . . . . . . . . . . . . . . . . T . . C . . . . . . . . . . . . [300]
Lycenchelys melanostomias          . . . . . T . . . . . G . T . . . . . C . . . . . C . . . . . [300]
Lycodapus microchir                . . . . . T . . . . . G . . . . . C . . . . . [300]
Lycodes albonotatus                . . . . . G . . . . . C . . . . . T . . . . . T . . C . . . . . [300]
Lycodes hubbsi                     C . . . . . C . . . . . G . . . . . C . . . . . T . . . . . G . . . [300]
Lycodes japonicus                  . . . . . T . . . . . T . . . . . A . . . . . T . . C . . . . . [300]
Lycodes matsubarai                 . . . . . T . . . . . T . . . . . T . . . . . T . . C . . . . . [300]
Lycodes nakamurai                  . . . . . G . . . . . T . . C . . . . . [300]
Lycodes ocellatus                  C . . . . . C . . . . . G . . . . . T . . . . . G . . . [300]
Lycodes pectoralis                 . . . . . G . . . . . T . . C . . . . . [300]
Lycodes raridens                   . . . . . C . . T . . . . . G . . . . . T . . . . . [300]
Lycodes sadoensis                  . . . . . T . . . . . G . . . . . A . . . . . C . . . . . [300]
Lycodes soldatovi                  . . . . . T . . . . . G . . [300]
Lycodes tanakae                    . . . . . C . . T . . . . . G . . . . . T . . . . . [300]
Lycodes teraoi                     . . . . . T . . . . . G . . . . . A . . . . . C . . . . . [300]
Lycodes toyamaensis                . . . . . T . . . . . G . . . . . C . . . . . [300]
Stichaeus nozawai                  . . . . . T . . A . . T . . . . . T . . . T . . . . C . . . . . T . . G . . C . . . . . C . . . . . G [300]
Zoarces americanus                 . . . . . C . . . . . G . . . . . T . . A . . A . . C . . . . . C . . . . . G . . G [300]
Zoarces elongatus                  . . . . . C . . . . . G . . . . . T . . A T . A . . C . . . . . C . . . . . [300]
Zoarces gilli                      . . . . . C . . . T . . . . . T . . T . . . . . A . . A . . . . . C . . . C . C . . . . . [300]

Bothrocara hollandi                A C A T G A A A C A T T G G C G T T A T C T T A T T A C T T C T C G T A A T A A T A A C A G C C T T C G T G G G C T A C [360]
Davidijordania poecillimon         . . . . . G . C . . T . . A . . T . . . C . T . . . . . T . G . . . . . T . . . . . [360]
Krusensterniella maculata          . . . . . C . . T . . A . . T . . . C . T . . . . . T . C . . . . . T . . . . . G . . . [360]
Lycenchelys albomaculata           . . . . . C . . T . . . . . T . . . C . . T . A . . . . . G . . . . . [360]
Lycenchelys aurantiaca             . . . . . C . . G . . A . . T . . . C . . T . G . . . . . G . . . . . C . . . . . T [360]
Lycenchelys melanostomias          . . . . . C . . . . . G . . . . . T . . . . . A . . . . . [360]
Lycodapus microchir                . . G . . . . . C . . G . . . . . T . . . C . . . . . A . . . . . [360]
Lycodes albonotatus                . . . . . C . . T . . . . . T . . . C . . T . A . . . . . [360]
Lycodes hubbsi                     . . . . . C . . T . . . . . . . . C . G T . A . . . . . T . . . [360]
Lycodes japonicus                  . . G . . . . . C . . T . . . . . T . . . C . G . . C . . . . . [360]
Lycodes matsubarai                 . . . . . [360]
Lycodes nakamurai                  . . . . . C . . . . . [360]
Lycodes ocellatus                  . . . . . C . . T . . . . . T . . . C . . T . A . . . . . T . . . [360]
Lycodes pectoralis                 . . . . . C . . . . . [360]
Lycodes raridens                   . . . . . G . . . . . [360]
Lycodes sadoensis                  . . G . . . . . C . . T . . C . . T . . . C . . . . . A . . . . . T . . . . . A . . . . . [360]
Lycodes soldatovi                  . . . . . G . . . . . [360]
Lycodes tanakae                    . . . . . G . . . . . [360]
Lycodes teraoi                     . . G . . . . . C . . T . . C . . T . . . C . . . . . A . . . . . A . . . . . [360]
Lycodes toyamaensis                . . G . . . . . C . . T . . . . . T . . . C . . . . . A . . . . . [360]
Stichaeus nozawai                  . . . . . G . A . . A . . . G . T C . . C . C . . . C . . T . . . . . G . . . . . T . . T . . . . . [360]
Zoarces americanus                 . . G . . . . . C . . T . . . G . T . . . C . T . . . . . T . . . . . T . . T . . . . [360]
Zoarces elongatus                  . . G . . . . . C . . T . . C G . T . . . C . C . . C . . T . . . . . G . . . . T . . . . . T . . C . . A . . . [360]
Zoarces gilli                      . . . . . T . . C . . T . . . . . T . . . C . C . . C . . T . . G . . . . . T . . . . . T . . A . . A . . . [360]

Bothrocara hollandi                G T T T T A C C C T G A G G A C A G A T G T C C T T T T G A G G G G C A A C C G T C A T C A C C A A C C T C C T C T C C [420]
Davidijordania poecillimon         . . C . . G . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . . . . T [420]
Krusensterniella maculata          . . C . . . . . . . . . . . . A . . A . . . . . G . . . . . . . . . T . . T . . . . . . . . . . . . T [420]
Lycenchelys albomaculata           . . . . . G . . . . . T [420]
Lycenchelys aurantiaca             . . . C . . . . . . . . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycenchelys melanostomias          . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A [420]
Lycodapus microchir                . . C . . . . . G . . . . . A . . . . . C . . . . . . . . . . . . . . . . . T . . . . . . . . . . . T [420]
Lycodes albonotatus                . . C C . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycodes hubbsi                     . . . . . [420]
Lycodes japonicus                  . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycodes matsubarai                 . . . . . A . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . T . . . . . T [420]
Lycodes nakamurai                  . . . . . T . . . . . T [420]
Lycodes ocellatus                  . . C . . . . . T [420]
Lycodes pectoralis                 . . . . . T . . . . . T [420]
Lycodes raridens                   . . C . . . . . G . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycodes sadoensis                  . . C C . . . . . G . . G . . . . . A . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycodes soldatovi                  . . . . . A . . . . . A . . . . . T [420]
Lycodes tanakae                    . . . . . G . . A . . A . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycodes teraoi                     . . C . . . . . G . . G . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [420]
Lycodes toyamaensis                . . C . . . . . G . . . . . C . . . . . A . . G . . . . . T . . . . . . . . . . . . . . . . . . T [420]
Stichaeus nozawai                  . . C C . G . . . . . G . . A . . A . . A . . . . . . . . . . . . . . . . . . . . A . . T . . . . . . . . . T [420]
Zoarces americanus                 . . G . . . . . T . . . . . A . . A . . . . . C . . G . . . . . T . . T . . T . . . . . . . . . . . . T [420]
Zoarces elongatus                  . . G . . . . . T . . . . . G . . A . . A . . . . . C . . . . . . . . . . . T . . T . . . . . . . . . . . . T [420]
Zoarces gilli                      . . C . . G . . . . . . . . . . A . . . . . C . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . T [420]

Bothrocara hollandi                G C A G T A C C C T A T G T C G G G A A T T C T C T T G T C C A A T G A A T C T G G G G G G G C T T C T C A G T C G A C [480]
Davidijordania poecillimon         . . . . . C . . . . . C A . . . . . C G . A . . C . . . . . G . . G . . . . . A . . C . . . . . [480]
Krusensterniella maculata          . . . . . G . . G . . C A . . . . . T . . . . . . . . . . . . G . . . . . . . . . T . . . . . . . . . . . . T . . . [480]
Lycenchelys albomaculata           . . . . . C . . A . . . . . . . . . . C . . . . . . . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycenchelys aurantiaca             . . . . . G . . . . . A . . . . A . . C . . C . . . . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycenchelys melanostomias          . . . . . A . . C . . . . . C . . . . . . . . . . . G . . G . . . . . A . . . . . . . . . . . . T . . . [480]
Lycodapus microchir                . . C . . G . . G . . . . . G . C G . . . . . C . . . . . . . . . G . . . . . A . . . . . . . . . . G . . . . . [480]
Lycodes albonotatus                . . . . . A . . C A . . . . . A . . C . . C . . . . . . . . . G . . . . . A . . . . . . . . . . . . T . . . . . [480]
Lycodes hubbsi                     . . . . . A . . . . A . . . . . A . . C . . . . . . . . . . . . G . . . . . A . . . . . . . . . . . . . . . . . [480]
Lycodes japonicus                  . . . . . A . . . . . C . . . . . C . . . . . . . . . G . . . . . A . . . . . . . . . . . . G . . . . . [480]
Lycodes matsubarai                 . . . . . G . . G . . . A . . . . . . C G . C . . . . . A . . . . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycodes nakamurai                  . . . . . A . . . . . G C . . . . . C . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycodes ocellatus                  . . . . . A . . . . A . . . . . A . . C . . . . . . . . . . . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycodes pectoralis                 . . C . . . . . A . . . A . . . . . . C . . . . . C . . . . . G . . G . . . . . . . . . . . . . . . . . . . . [480]
Lycodes raridens                   . . . . . T . . A . . . A . . . . . . . . . . . . . T . . . . G . . . . . A . . . . . . . . . . . . . . . . . [480]
Lycodes sadoensis                  . . C . . G . . A . . . . . . . . . . C . . G . . C . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycodes soldatovi                  . . C . . . . . A . . . . A . . . . . . C G . . . . . . . . . G . . G . . T . . A . . . . . . . . . . . T . . T [480]
Lycodes tanakae                    . . . . . A . . . . A . . . . . . . . . . . . . . . . . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycodes teraoi                     . . C . . . . . A . . . . . . . . . . C . . G . . C . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Lycodes toyamaensis                . . C . . G . . A . . . . . . . . . . C G . G . . C . . . . . G . . G . . . . . A . . . . . . . . . . . . . . . [480]
Stichaeus nozawai                  . . . . . A . . C A . . . . . A . . C G . A . . . . . T . . . . . G . . . . . A . . . . . . . . . . C . . . . . T [480]
Zoarces americanus                 . . . . . C . . G . . C A . . . . . C . . . . . . . . . G . . T . . A . . A . . T . . . . . G . . . . . . [480]
Zoarces elongatus                  . . . . . C A . . . . . C . . . . . . . . . . . . . . . A . . T . . . . . . . . . . . . . . . . . T [480]
Zoarces gilli                      . . C . . . . . C A . T . . A . . . . . . . . . . . . G . . . . . A . . . . . . . . . . . . G . . . . . [480]
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Cytochrome *b* (*cytb*)

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Bothrocara hollandi           A A C G C T A C G C T A A C C C G A T T T T T T G C C T T T C A T T T C C T C T T C C C C T T T G T T A T T G C A G G C [540]
Davidijordania poecillimon    . . . . . C . . C . . G . . . . . G . . . . . C . . . . . C . . C . . . . . . . . . . . . . . . . . C . . . . C . . C . . . [540]
Krusensterniella maculata     . . . . . C T . . . . . C . . C . . . . . . . . . . . . . . . . . . . . . C . . . . . [540]
Lycenchelys albomaculata      . . . . . C . . . . . T . . . . . A . . . . . C . . . . . [540]
Lycenchelys aurantiaca        . . . . . C . . . . . T . . . . . G . . . A . . . . . [540]
Lycenchelys melanostomias     . . . . . C . . . . . T . . . . . A . . . . . T [540]
Lycodapus  microchir          . . . . . C . . A . . . . . C . . . . . T . . . . . A . . . . . [540]
Lycodes albonotatus           . . . . . C . . . . . T . . . . . A . . . . . [540]
Lycodes hubbsi                . . . . . A . . C . . . . . [540]
Lycodes japonicus             . . . . . A T . . . . . T . . . . . . . . . . . . . . . . . . T [540]
Lycodes matsubarai            . . . . . A . . . . . T [540]
Lycodes nakamurai             . . . . . C . . . . . A . . . . . T [540]
Lycodes ocellatus             . . . . . T . . . . . G . . . . . [540]
Lycodes pectoralis            . . . . . A . . . . . T [540]
Lycodes raridens              . . . . . A . . . . . T [540]
Lycodes sadoensis             . . . . . C . . A . . . . . T . . . . . A . . . . . [540]
Lycodes soldatovi             . . . . . C . . . . . A . . . . . T [540]
Lycodes tanakae               . . . . . A . . . . . C . . . . . [540]
Lycodes teraoi                . . . . . C . . A . . . . . T . . . . . A . . . . . [540]
Lycodes toyamaensis           . . . . . C . . A . . G . . . . . G . . . . . T . . . . . A . . . A . . . . . [540]
Stichaeus nozawai             . . . . . C . . T . . . . . C . . . . . T . . C . . C . . T . . . . . A . . C . . . . . . . . . . T [540]
Zoarces americanus            . . . . . A . . . . . C . . . . . C . . C . . . . . G . . . . . [540]
Zoarces elongatus             . . . . . A . . . . . C . . . . . C . . C . . . . . C . . . . . [540]
Zoarces gilli                 . . . . . C . . A . . . . . C . . . . . C . . . . . T . . . . . A . . . . . G [540]

Bothrocara hollandi           G C C A C T T T A G T A C A C C T G C T C T T T C T A C A T C A A G T G G G C T C A A C A A A C C C C C T G G G C T T A [600]
Davidijordania poecillimon    . . . . . G . . . . . T . . . . C . . . . . A C . . . . . T . . . . . G [600]
Krusensterniella maculata     . . . . . C . . G . . C . . T . . C . . . A C . . . . . A T . . . . . T . . . T . . . [600]
Lycenchelys albomaculata      . . . . . T . . T . . C . . . . . C . . G A C . . . T . . . . . T . . . . . T . . . C . . [600]
Lycenchelys aurantiaca        . . . . . G . . G . . . . T . . . . C . . G . . C . . G A C . . . . . T . . . . . T . . . . C . . G [600]
Lycenchelys melanostomias     . . . . . G . . . . . A C A . . . . . A . . . . . [600]
Lycodapus  microchir          . . . . . T . . T . . C . . . . . C . . . A C . . . . . C . . . . . T . . T C . G [600]
Lycodes albonotatus           . . . . . T . . . . . C . . . . . C . . G A C . . . . . C . . . . . A . . . C . . [600]
Lycodes hubbsi                . . . . . C . . . . . T . . T . . C . . . . . C . . G A C . . . . . T . . T . . G . . T . . . C . . [600]
Lycodes japonicus             . T . . . A C . . . . . T . . T . . C . . . . . C . . . A C A . . . . . C . . . . . A . . . C . G [600]
Lycodes matsubarai            . . . . . G . . . . . G . . . . . G A C A . . . . . T . . T . . . [600]
Lycodes nakamurai             . . . . . G . . . . . T . . A . . . . . A C A . . . . . T . . . . . [600]
Lycodes ocellatus             . . . . . T . . T . . C . . . . . C . . G A C . . . . . T . . T . . G . . T . . . C . . [600]
Lycodes pectoralis            . . . . . G . . . . . T . . A . . . . . A C A . . . . . T . . . . . [600]
Lycodes raridens              . . . . . C . . . . . G . . . . . T . . . . . A C A . . . . . T . . T . . . [600]
Lycodes sadoensis             . . . . . T . . T . . C . . . . . C . . . A C . . . . . C . . T . . . . . C . . . C . G [600]
Lycodes soldatovi             . . . . . C . . C . . G . . . . . T . . . . . G . . . . . A C . . . . . T . . . . . [600]
Lycodes tanakae               . . . . . A . . . . G . . . . . T . . . . . A C A . . . . . T . . T . . . [600]
Lycodes teraoi                . . . . . T . . T . . C . . . . . C . . . A C . . . . . C . . . . . C . . . C . G [600]
Lycodes toyamaensis           . . . . . T . . T . . C . . . . . C . . . A C . . . . . C . . . . . T . . G C . G [600]
Stichaeus nozawai             . . . . . A . G . . T . . . . . T . . T . . C . . T . . C . . . A C C . . . . . A C . . . . . T . . T . . . [600]
Zoarces americanus            . . . . . C . . . . . T . . T . . C . . C . . C . . . A C . . . . . A T . . . . . T . . . . . G [600]
Zoarces elongatus             . . . . . T . . T . . C . . C . . C . . . A C A . . . . . A C . . . . . T . . . C . G [600]
Zoarces gilli                 . . . . . A A . . . . T . . T . . C . . . . . C . . T . . C . . . A C A . . . . . A C . . . . . C . . G C . G [600]

Bothrocara hollandi           A A C T C A G A T G C T G A C A A A A T C T C C T T C C A T C C C T A C T T T T C G T A T A A A G A T C T T C T T G G C [660]
Davidijordania poecillimon    . . . . . T . . . . . C . . . . . C . . . . . C . . . . . C . . C . . . . . [660]
Krusensterniella maculata     . . T . . . . . T . . G G . . . . A . . . . . C . . . . . T . . . . . G . . . . . [660]
Lycenchelys albomaculata      . . . . . A . . . . . C . . . . . C . . . . . [660]
Lycenchelys aurantiaca        . . . . . G . . . . . A . . . . . C . . . . . C . . . . . G [660]
Lycenchelys melanostomias     . . . . . A . . . . . G . . . . . [660]
Lycodapus  microchir          . . . . . G . . . . A . . . . . C . . . . . A . . . . . C . . . . . [660]
Lycodes albonotatus           . . . . . A . . . . . C . . . . . C . A . . . . . [660]
Lycodes hubbsi                . . . . . C . . . . . C . . . . . [660]
Lycodes japonicus             . . . . . C . . . . . G . . C . . C . . G . . . [660]
Lycodes matsubarai            . . . . . T . . . . . A . . . . . C . . . . . C . . . . . [660]
Lycodes nakamurai             . . . . . C . . . . . C . . . . . [660]
Lycodes ocellatus             . . . . . C . . . . . C . . . . . [660]
Lycodes pectoralis            . . . . . C . . . . . A . . . . . C . . A . . . . . [660]
Lycodes raridens              . . . . . A . . . . . C . . . . . G . . . . . [660]
Lycodes sadoensis             . . . . . A . . . . . C . . . . . C . . . . . [660]
Lycodes soldatovi             . . . . . A . . . . . C . . . . . C . . . . . [660]
Lycodes tanakae               . . . . . C . . T . . . . . G . . . . . [660]
Lycodes teraoi                . . . . . A . . . . . C . . . . . C . . . . . [660]
Lycodes toyamaensis           . . . . . G . . . . A . . . . . C . . . . . A . . . . . C . . . . . [660]
Stichaeus nozawai             . . T . . . . . T . . . . . T . . T . . . . . C . . . . . T . . . . . G . . . T . G . . A . . . [660]
Zoarces americanus            . . . . . T . . . . . A . . . . . C . . . . . A . . . . . C . . . . . [660]
Zoarces elongatus             . . T . . . . . C . . . . . G . . . . . A . . T . . C . . . . . C . . C . . . . . C . . . [660]
Zoarces gilli                 . . T . . . . . C . T . . . . . G . . T . . C . . T . . . . . C . . . . . G . . C . . C . . C . . . [660]

Bothrocara hollandi           T T T G C A G C C T T A G T C C T T G G C C T T A C A G C C C T A G C A C T A T T C T T C C C C A A C C T C [714]
Davidijordania poecillimon    . . . . . T . . . . . T A . . . . T . . . . . G T . C . . . . . T . C A . . . . . [714]
Krusensterniella maculata     . . C A . . . . T . . . . . A . . . . A . . . . . C . . . . . T . C A . . T . . T . . . [714]
Lycenchelys albomaculata      . . . . . T . . . . . A . C . . . . . G . . . . . T C . T . . . . . [714]
Lycenchelys aurantiaca        . . . . . A . . A . C . . . . . C . . . . . G . . . . . T . . . . . [714]
Lycenchelys melanostomias     . . . . . G . . . . . A . . . . G . . . . . T . . . . . A [714]
Lycodapus  microchir          . . . . . T . . . . . A . C . . . . . C . . . . . C . . . . . [714]
Lycodes albonotatus           . . . . . T . . . A . . A . C . . . . . G . . . . . T . . . . . [714]
Lycodes hubbsi                . . . . . A . . . . . A . C . . . . . G . . C . . . . . [714]
Lycodes japonicus             . . . . . T . . . . . C . . . . . C . . . . . C . . . . . T . . . . . [714]
Lycodes matsubarai            . . . . . G A . . . . . A . . . A . . . . . T . . . . . T . . . . . [714]
Lycodes nakamurai             . . . . . G . . . . . T . A . . . . . C . . . T . . . . . [714]
Lycodes ocellatus             . . . . . A . . . . . A . C . . . . . G . . C . . . . . T . . . . . [714]
Lycodes pectoralis            . . . . . G . . . . . A . . . . . C . . . T . . . . . [714]
Lycodes raridens              . . . . . G . . . . . A . . . . . G . . . . . C . . . . . [714]
Lycodes sadoensis             . . . . . T . . G . . . . . C . . . . . C . . . . . G . . . . . C . . . . . [714]
Lycodes soldatovi             . . . . . G A . . . . . A . . . . T . . . . . [714]
Lycodes tanakae               . . . . . G . . . . . A . . . . . [714]
Lycodes teraoi                . . . . . T . . G . . . . . C . . . . . C . . . . . G . . . . . C . . . . . [714]
Lycodes toyamaensis           . . . . . T . . G . . . A . C . . . . . C . . . . . C . . . . . [714]
Stichaeus nozawai             . . . . . C . G . . A A . . . . . C . . T . . . . . T . C A . . A . . T . . . [714]
Zoarces americanus            . . . . . T C . . A . A G . . . . . C . . . . . C . . . . . T . . . . . [714]
Zoarces elongatus             . . . . . T C . . . . . A A C . . . . . C . . . . . C . . G . . . . . C . . . . . [714]
Zoarces gilli                 . . . . . T C . . . . C A G . C . . . . . C . . G . . . . . C T . . . . . [714]
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Cytochrome *c* oxidase subunit I (COI)

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Bothrocara hollandi                G T G G G C A C A G C T C T A A G T C T A C T C A T T C G A G C A G A G C T A A G C C A A C C C G G C G C C C T C T T G [ 60]
Davidijordania poecillimon         . . A . . . . . . . . . T . . . . C . . C . . . . . . . . . . . . G . . . . . . . . . . . . G . . . . . . . . . . . . . [ 60]
Krusensterniella maculata          . . A . . . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycenchelys albomaculata           . . . . . . . . . . . . . . . G . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycenchelys aurantiaca             . . . . . . . . . . . . . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . T . . . . . C . . [ 60]
Lycenchelys melanostomias          . . . . . . . . . . . . . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodapus  microchir               . . . . . . . . . . . . . . . . . . C . . G . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes albonotatus                . . . . . . . . . . . C . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes hubbsi                     . . A . . . . . . . . . . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycodes japonicus                  . . A . . . . . . . . C . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycodes matsubarai                 . . T . . T . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . T C . . [ 60]
Lycodes nakamurai                  . . T . . T . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycodes ocellatus                  . . . . . . . . . . . . . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . T . . . . . C . . [ 60]
Lycodes pectoralis                 . . T . . T . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycodes raridens                   . . T . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycodes sadoensis                  . . . . . . . . . . C . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes soldatovi                  . . T . . T . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . T C . . [ 60]
Lycodes tanakae                    . . A . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]
Lycodes teraoi                     . . . . . T . . . . . C . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes toyamaensis                . . . . . . . . . . C . . . . . . C . . C . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Stichaeus nozawai                  . . A . . . . . . . . . . . . . . . C . . C . . . . . C . . . . . . . . . . . . . . . G . . . . . . . . . . . T . . A [ 60]
Zoarces americanus                 . . T . . . . . . . . C . . . . . . C . . C . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Zoarces elongatus                  . . T . . . . . . . . C . . . . . . C . . C . . . . . . . . . . . G . . . . . . G . . . . . . A . . . . . . . . . . . [ 60]
Zoarces gilli                      . . T . . . . . . . . C . . G . . C . . C . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . C . . [ 60]

Bothrocara hollandi                G G G G A C G A C C A A A T T T A C A A T G T C C T T G T T A C A G C G C A T G C G T T C G T A A T A A T T T T C T T T T [120]
Davidijordania poecillimon         . . A . . T . . . . . . . . . . . . T . . . . . . A . . . . C . . . . . A . . . . . . . . . . . . . . . . . . . . . . [120]
Krusensterniella maculata          . . C . . T . . . . . G . . . . . . T . . . . . T A . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . [120]
Lycenchelys albomaculata           . . . . . . . . . . G . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycenchelys aurantiaca             . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . A . . . . . A . . . . . . . . . . . . . . [120]
Lycenchelys melanostomias          . . . . . . . . . . G . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodapus  microchir               . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes albonotatus                . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes hubbsi                     . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes japonicus                  . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes matsubarai                 . . . . . T . . . . . . . . . . . . T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes nakamurai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes ocellatus                  . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes pectoralis                 . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes raridens                   . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes sadoensis                  . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . A . . C . . A . . . . . . . . . . . . . . . [120]
Lycodes soldatovi                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes tanakae                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Lycodes teraoi                     . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . C . . . . . A . . C . . A . . . . . . . . . . . . . . . [120]
Lycodes toyamaensis                . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [120]
Stichaeus nozawai                  . . . . . . . . . . . . . . . . . . T . . C . . A A . . . . . . . . G . . C . . . . . . . . . . . . . . . . . . . . . . [120]
Zoarces americanus                 . . . . . T . . . . . . . . . C . . . . . . . . . A . . . . . . . . . . A . . . . . . . . . . . . . . . . . . C . . . . . . [120]
Zoarces elongatus                  . . . . . . . . . . . . . . . . . . T . . . . . . A . . . . . . . . . . A . . . . . . . . . . . . . . . . . . C . . . . . . [120]
Zoarces gilli                      . . . . . . . . . . . . . . . G . . . . . . . . . . T A . . . . . C . . . . . A . . . . . . . . . T . . . . . . C . . . . . . [120]

Bothrocara hollandi                A T A G T A A T A C C A A T T A T G A T C G G G G G G G T T T G G G A A C T G A C T T G T G C C C T T A A T A A T C G G G [180]
Davidijordania poecillimon         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . A . . . . . G . . . . . T . . . . . C . . C . G . . . . . . . . . . . [180]
Krusensterniella maculata          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . A . . . . . G . . . . . T . . . . . . . . . . . . . A [180]
Lycenchelys albomaculata           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . C . . . . . A . . . . . G . . . . . A . . . . . . . . . . . . . . . [180]
Lycenchelys aurantiaca             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . A . . . . . G . . . . . A . . . C . G . . . . . T . . . [180]
Lycenchelys melanostomias          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . G . . . . . . . . . . . . . . . . . [180]
Lycodapus  microchir               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . A . . . . . G . . . . . A . . . . . . . . . . . T . . . [180]
Lycodes albonotatus                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . C . . A . . . . . G . . . . . . . . . . . . . . . . . T . . . [180]
Lycodes hubbsi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . A . . . . . G . . C . . . . . . . . . . . . . . . . . [180]
Lycodes japonicus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . A . . T . . . . . A . . . . . G . . C . . A . . . . . G . . . . . T . . A [180]
Lycodes matsubarai                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . T . . . . . A . . . . . G . . C . . . . . . . . . . . . . T . . . [180]
Lycodes nakamurai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . T . . . . . A . . . . . G . . C . . . . . . . . . . . . . T . . . [180]
Lycodes ocellatus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . C . . . . . A . . . . . G . . C . . A . . . . . G . . . . . T . . . [180]
Lycodes pectoralis                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . T . . . . . A . . . . . G . . C . . . . . . . . . . . . . T . . . [180]
Lycodes raridens                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . T . . . . . A . . . . . G . . C . . A . . . . . . . . . . . T . . . [180]
Lycodes sadoensis                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . C . . A . . . . . G . . . . . . . . . . . . . . . . . . . . . . [180]
Lycodes soldatovi                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . T . . . . . A . . . . . G . . C . . A . . . . . . . . . . . T . . . [180]
Lycodes tanakae                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . T . . . . . A . . . . . G . . C . . . . . C . . . . . . . . . T . . . [180]
Lycodes teraoi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . C . . A . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . [180]
Lycodes toyamaensis                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . C . . A . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . [180]
Stichaeus nozawai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . A . . C . . A . . . . . G . . . A . C . . G . . . . . G . . . . . A [180]
Zoarces americanus                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . T . . A . . . . . A . . . . . G . . . . . C . . . . . . . . . G . . . . . A [180]
Zoarces elongatus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . A . . . . . C . . A . . . . . G . . . A . C . . . . . . . . . G . . . . . . [180]
Zoarces gilli                      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . C . . A . . . . . G . . . . . C . . . . . . . . . G . . . . . . [180]

Bothrocara hollandi                G C C C C G G A C A T A G C A T T T C C C C G A A T A A A C A A C A T G A G C T T T T T G A C T C C T T C C C C C G T C T [240]
Davidijordania poecillimon         . . . . . C . . . T . . G . . C . . C . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . G . . . . . C . . . . . . . . . . [240]
Krusensterniella maculata          . . . . . A . . . . . G . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . C . . . . . A . . . . [240]
Lycenchelys albomaculata           . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . T . . A . . . [240]
Lycenchelys aurantiaca             . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycenchelys melanostomias          . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . C . . . [240]
Lycodapus  microchir               . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes albonotatus                . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes hubbsi                     . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C [240]
Lycodes japonicus                  . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes matsubarai                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes nakamurai                  . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes ocellatus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes pectoralis                 . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes raridens                   . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes sadoensis                  . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . C [240]
Lycodes soldatovi                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes tanakae                    . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Lycodes teraoi                     . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . C [240]
Lycodes toyamaensis                . . . . . A . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . [240]
Stichaeus nozawai                  . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C G . . [240]
Zoarces americanus                 . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . A . . . . . . . . . . . . . T . . A . . . [240]
Zoarces elongatus                  . . . . . A . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Zoarces gilli                      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . A . . . C [240]
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Cytochrome *c* oxidase subunit I (COI)

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Bothrocara hollandi                T T T C T T C T C C T C C T T G C C T C T T C C G G G G G T A G A A G C G G G C G C T G G G A C G G G G G T G A A C A G T T [300]
Davidijordania poecillimon         . . . . . C . . T . . . . . . . . . . . . . . . G . . . . . G . . G . . . . . A . . . . . . . . . A . . . . . . . . . . . C [300]
Krusensterniella maculata          . . . . . C . . . . . . . . . . . . . . . . . . G . . . . . G . . . . . . . . . G . . . . . C . . A . . . . . . . . . . . [300]
Lycenchelys albomaculata           . . . . . T . . . . . G . . . . . . . . . A . . T . . . . . . . . A . . A . . . . . . . . . . . [300]
Lycenchelys aurantiaca             C . . . . C . . . . . . . . . . . . . . . A . . . . . G . . G . . . . . . . . . . . A . . . . . . . . . T . . . [300]
Lycenchelys melanostomias          . . . . . T . . . . . G . . . . . . . . . G . . . . . G . . . . . . . . . . . C . . . . . G . . T . . . [300]
Lycodapus microchir                . . . . . A . . . . . G . . G . . A . . T . . . . . . . . A . . . . . . . . . . . . . . . . . . . [300]
Lycodes albonotatus                . . . . . T . . . . . G . . . . . . . . . T . . A . . . . . C . . . . . G . . . . . . . . . . . [300]
Lycodes hubbsi                     . . . . . T . . . . . A . . . . . . . . . T . . C . . . . . A . . . . . G . . . . . . . . . . . [300]
Lycodes japonicus                  . . . . . C . . . . . T . . . . . G . . . . . . . G . . . . . G . . . . . C . . . . . . . . . . . [300]
Lycodes matsubarai                 . . C . . . . . . . . . . . . . . . G . . . . . . . G . . . . . T . . . . . A . . . . . . . . . . C [300]
Lycodes nakamurai                  . . . . . G . . . . . G . . . . . T . . . . . A . . A . . . . . . . . . . [300]
Lycodes ocellatus                  . . . . . C . . . . . T . . . . . G . . . . . G . . . . . G . . . . . C . . . . . . . . . . C [300]
Lycodes pectoralis                 . . . . . G . . . . . G . . . . . T . . . . . A . . A . . . . . . . . . . [300]
Lycodes raridens                   . . . . . G . . . . . G . . G . . . . . T . . . . . A . . A . . . . . . . . . . [300]
Lycodes sadoensis                  . . . . . C . . . . . T . . . . . G . . . . . G . . A . . . . . . . . . G . . . . . . . [300]
Lycodes soldatovi                  . . . . . A . . T . . . . . G . . . . . . . . . A . . . . . . . . . . . . . . . . . . . [300]
Lycodes tanakae                    . . . . . G . . . . . G . . . . . T . . . . . A . . A . . G . . . . . . . [300]
Lycodes teraoi                     . . . . . T . . . . . G . . . . . A . . . . . . . . . G . . . . . . . [300]
Lycodes toyamaensis                . . . . . T . . . . . G . . . . . T . . A . . . . . C . . . . . G . . . . . . . [300]
Stichaeus nozawai                  . . . . . G . . T . . . . . T . . . . . G . . T . . A . . . . . . . . . G . . . . . . . [300]
Zoarces americanus                 . . . . . C . . A . . . . . G . . A . . A . . . . . C . . . . . A . . . . . . . . . C [300]
Zoarces elongatus                  . . . . . C . . G . . . . . G . . G . . A . . A . . . . . C . . . . . . . . . . . . . C [300]
Zoarces gilli                      . . . . . C . . . . . T . . . . . G . . . . . A . . . . . C . . A . . . . . . . . . . [300]

Bothrocara hollandi                T A C C C C C C T C T C T C C G G T A A C T T A G C C C A T G C G G G G G C C T C C G T T G A T T T A A C A A T C T T C [360]
Davidijordania poecillimon         . . . . . G . . T . . T . . . . . C . G . . . . . C . . . . . T . . . . . . . . . . . . . . . . . . . [360]
Krusensterniella maculata          . . . . . G . . . . . T . . G . . . C . . . . . C . . A . . . . . T . . G . . . . . . . . . . . . T [360]
Lycenchelys albomaculata           . . . . . T . . T . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . [360]
Lycenchelys aurantiaca             . . . . . T . . T . . . . . G . . . . C . . A . . . . . . . . . . . . . . C . . . . . . . . . . . [360]
Lycenchelys melanostomias          . . T . . . . . T . . T . . . . . C . G . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . [360]
Lycodapus microchir                . . . . . T . . G . . . . . . . . . . C . . . . . . . . . . . . . . . C . . . . . . . . . . [360]
Lycodes albonotatus                . . . . . T . . T . . C . . . C . G . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes hubbsi                     . . . . . T . . T . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes japonicus                  . . . . . T . . T . . C . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes matsubarai                 . . . . . T . . . . . A . . . . . T . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes nakamurai                  . . . . . T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . T . . T [360]
Lycodes ocellatus                  . . . . . T . . T . . C . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes pectoralis                 . . . . . T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . T . . T [360]
Lycodes raridens                   . . . . . T . . . . . A . . A . . . . . . . . . . . . . . . G . . . . . . . . . [360]
Lycodes sadoensis                  . . . . . T . . T . . C . . . C . G . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes soldatovi                  . . . . . T . . C . . . . . . . . . . . . . . A . . A . . . . . . . . . . . . . . . . . [360]
Lycodes tanakae                    . . . . . T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . [360]
Lycodes teraoi                     . . . . . T . . T . . C . . . C . G . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes toyamaensis                . . . . . T . . T . . C . . . C . G . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . [360]
Stichaeus nozawai                  . . . . . T . . C . . T . . G . . C . . T . . . . . . . . . C . . . . . T . . A . . . . . G . . . . . . . T [360]
Zoarces americanus                 . . . . . T . . T . . T . . G . . . . . G . . . . . . . . . A . . T . . T . . . . . . . . . . . . . . . [360]
Zoarces elongatus                  . . . . . T . . . . . T . . T . . T . . A . . . . . G . . . . . . . . . . . . . T . . T . . . . . . . . . . . T [360]
Zoarces gilli                      . . T . . T . . . . . T . . T . . A . . . . . G . . . . . C . . . . . . . . . T . . . . . G . . . . . . . . . . . [360]

Bothrocara hollandi                T C C C T T T C A C T T T A G C A G G G G T C T C T T C A A T C C T C G G G G G C A A T T A A C T T C A T T A C G A C C A T C [420]
Davidijordania poecillimon         . . . . . G . . . A . T . . C . . . . . T . . T . . A . . . . . C . . . . . . . . . . . A . . . . . T [420]
Krusensterniella maculata          . . T . . . . . G . . . A . T . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . T [420]
Lycenchelys albomaculata           . . . . . A . T . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . A . . . . . . [420]
Lycenchelys aurantiaca             . . . . . A . . . . . T . . . . . . . . . . . . . . . . . . . . . A . . . . . . [420]
Lycenchelys melanostomias          . . . . . A . T . . . . . T . . . . . . . . . . . T . . . . . A . . . . . . [420]
Lycodapus microchir                . . . . . C . . . . . T . . . . . . . . . . . T . . . . . . . . . . . . . . [420]
Lycodes albonotatus                . . . . . A . T . . . . . T . . . . . . . . . . . . . . . . . A . . . . . . [420]
Lycodes hubbsi                     . . . . . G . . A . T . . . . . . . . . . . T . . . . . A . . T . . . [420]
Lycodes japonicus                  . . . . . C . . . . . A . . . . . . . . . . . . . . . . . . . . . A . . . . . . [420]
Lycodes matsubarai                 . . . . . C . . . . . A . T . . . . . . . . . . . . A . . . . . T . . . . . . . [420]
Lycodes nakamurai                  . . . . . A . T . . . . . T . . . . . . . . . . . T . . . . . [420]
Lycodes ocellatus                  . . . . . C . . . . . A . . . . . . . . . . . . . . . . . . . . . A . . . . . . [420]
Lycodes pectoralis                 . . . . . A . T . . . . . T . . . . . . . . . . . T . . . . . . . . . . . . . . [420]
Lycodes raridens                   . . . . . C . . . . . A . T . . . . . . . . . . . T . . T . . . . . . . . . . [420]
Lycodes sadoensis                  . . . . . A . T . . . . . T . . . . . . . . . . . . . . . . . A . . . . . . [420]
Lycodes soldatovi                  . . . . . C . . C . . . . . A . . . . . . . . . . . T . . . . . . . . . . . [420]
Lycodes tanakae                    . . T . . C . . C . . . . . A A . T . . . . . . . . . . T . . T . . . . . . [420]
Lycodes teraoi                     . . . . . A . T . . . . . T . . . . . . . . . . . . . . . . . A . . . . . . [420]
Lycodes toyamaensis                . . . . . A . T . . . . . T . . . . . . . . . . . . . . . . . A . . . . . . [420]
Stichaeus nozawai                  . . T . . . . . T C . . . T . . A A . T . . . . . . . T . . A . . . . . . C . . T . . T . . . . . A . . T . . T [420]
Zoarces americanus                 . . . . . G . . . A . . . . . . . G . . . . . . . . . A . . . . . . . . . . . A . . . . . . [420]
Zoarces elongatus                  . . . . . C . . . C . . . . G . . A A . . . . . G . . . . . . . . . . . T . . . . . A . . . . . . [420]
Zoarces gilli                      . . . . . G . . . . . . . . G . . A A . T . . . . . . . . . . . . . . A . . . . . . T . . . . . C . . A . . . . . . [420]

Bothrocara hollandi                A T T A A C A T G A A G C C C C C T G C G A T C T C T C A G T A C C A G A C A C C C C T C T T C G T C T G G T C C G T G [480]
Davidijordania poecillimon         . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . [480]
Krusensterniella maculata          . . . . . A . . . . . G . . C . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . A . . T . . A [480]
Lycenchelys albomaculata           . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . A [480]
Lycenchelys aurantiaca             . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . A . . . [480]
Lycenchelys melanostomias          . . C . . . . . T . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . G . . A [480]
Lycodapus microchir                . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A [480]
Lycodes albonotatus                . . C . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . G . . A [480]
Lycodes hubbsi                     . . . . . [480]
Lycodes japonicus                  . . C . . . . . C . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . A . . A [480]
Lycodes matsubarai                 . . . . . [480]
Lycodes nakamurai                  . . . . . T . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes ocellatus                  . . . . . A . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . A . . A [480]
Lycodes pectoralis                 . . . . . A . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes raridens                   . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes sadoensis                  . . C . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . G . . . [480]
Lycodes soldatovi                  . . . . . [480]
Lycodes tanakae                    . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes teraoi                     . . C . . . . . A . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . G . . . [480]
Lycodes toyamaensis                . . C . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . G . . C [480]
Stichaeus nozawai                  . . . . . A . . . . . C . . . . . . . . . . . T . . A . . . . . . . . . A . . T . . T . . A . . G . . A [480]
Zoarces americanus                 . . . . . A . . A . . . . . T . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . A . . T . . C [480]
Zoarces elongatus                  . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . T . . C [480]
Zoarces gilli                      . . C . . . . . A . . . . . T . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . A . . T . . C [480]
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Cytochrome *c* oxidase subunit I (COI)

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Bothrocara hollandi                C T T A T T A C A G C A G T C C T A C T C C T C C T T T C T C T C C C C G T C C T T G C A G C C G G T A T T A C C A T G [540]
Davidijordania poecillimon         . . C . . C . . G . . C . . . . . T . A . . . . . C . . . . . T . . G . . . . . . . G . . C . . C . . . . . A [540]
Krusensterniella maculata          . . . . . C . . G . . C . . . . . A . . . . . A . G . . . . . G . . . . . T . . . . . C . . . . . [540]
Lycenchelys albomaculata           . . . . . G . . G . . . . . G . . . . . C . . . . . C . . . . . T . . . . . C . . . . . [540]
Lycenchelys aurantiaca             . . . . . G . . G . . . . . C . . . . . C . . . . . C . . . . . T . . G . . C . . . . . [540]
Lycenchelys melanostomias          . . . . . G . . G . . T . . . . . C . . G . . . . . G . . . . . C . . . . . [540]
Lycodapus  microchir               . . . . . G . . . . . C . . . . . T . . . . . [540]
Lycodes albonotatus                . . . . . C . . G . . G . . . . . T . . . . . C . . . . . [540]
Lycodes hubbsi                     . . . . . G . . . . . G . . . . . C . . . . . T . . . . . C . . . . . A [540]
Lycodes japonicus                  . . A . . C . . G . . G . . . . . G . . . . . C . . . . . T . . . . . . . . . . [540]
Lycodes matsubarai                 . . . . . G . . . . T . . . . . T . . . . . C . . G . . . . . T . . A . . . . . A [540]
Lycodes nakamurai                  . . C . . . . . G . . G . . T . . . . . T . . . . . C . . . . . T . . A . . . . . . [540]
Lycodes ocellatus                  . . . . . C . . G . . G . . . . . G . . . . . C . . . . . T . . . . . C . . . . . [540]
Lycodes pectoralis                 . . C . . . . . G . . G . . T . . . . . T . . . . . C . . . . . T . . A . . . . . . [540]
Lycodes raridens                   . . C . . . . . G . . G . . . . . T . . . . . C . . . . . T . . A . . . . . . [540]
Lycodes sadoensis                  . . . . . C . . G . . G . . . . . C . . C . . . . . T . . . . . C . . . . . [540]
Lycodes soldatovi                  . . C . . . . . G . . . . . T . . . . . T . . . . . C . . . . . T . . A . . . . . . [540]
Lycodes tanakae                    . . C . . . . . G . . G . . . . . T . . . . . C . . . . . A . . . . . [540]
Lycodes teraoi                     . . . . . C . . G . . G . . . . . C . . C . . . . . T . . . . . C . . . . . [540]
Lycodes toyamaensis                . . . . . C . . G . . G . . . . . T . . . . . C . . . . . [540]
Stichaeus nozawai                  T . A . . . . . T . . . . . T . . G . . T . . C . . C . . . . . T . . . . . C . . . . . G . . A [540]
Zoarces americanus                 . . . . . C . . G . . T . . . . . G . . . . . C . . C . . . . . G . . C . . . . . C . . A . . . [540]
Zoarces elongatus                  . . . . . C . . G . . T . . . . . A . . . . . C . . C . . . . . G . . C . . . . . A . . . [540]
Zoarces gilli                      . . . . . C . . G . . T . . . . . A . . . . . C . . C . . . . . A . . G . . . . . C . . C . . G . . . [540]

Bothrocara hollandi                C T C C T G A C A G A T C G T A A C C T T A A T A C C A C C T T C T T C G A C C C C G C C G G A G G A G G A G A C C C A [600]
Davidijordania poecillimon         . . . . . C . . C . . . . . T . . . . . G . . . . . G [600]
Krusensterniella maculata          . . . . . A . . . . . C . . . . . C . . T . . . . . G . . G . . . . . T [600]
Lycenchelys albomaculata           . . . . . T . . . . . C . . . . . G . . . . . [600]
Lycenchelys aurantiaca             . . . . . C . . . . . C . . . . . C . . . . . G . . . . . G . . . . . [600]
Lycenchelys melanostomias          . . T . . A . . . . . C . . C . . . . . T . . . . . G . . . . . G . . . . . [600]
Lycodapus  microchir               . . . . . T . . . . . C [600]
Lycodes albonotatus                . . . . . A . . . . . C . . . . . T . . . . . G . . . . . . . . . . [600]
Lycodes hubbsi                     . . . . . C . . . . . C . . . . . G . . C . . G . . . . . C [600]
Lycodes japonicus                  . . . . . C . . . . . C . . . . . G . . . . . . . . . . C [600]
Lycodes matsubarai                 . . . . . C . . . . . G . . . . . . . . . . [600]
Lycodes nakamurai                  . . . . . C . . . . . G . . . . . . . . . . [600]
Lycodes ocellatus                  . . . . . C . . . . . C . . . . . T . . . . . G . . . . . G . . . . . C [600]
Lycodes pectoralis                 . . . . . C . . . . . G . . . . . . . . . . [600]
Lycodes raridens                   . . . . . C [600]
Lycodes sadoensis                  . . . . . A . . . . . C . . . . . T . . . . . G . . . . . . . . . . [600]
Lycodes soldatovi                  . . . . . [600]
Lycodes tanakae                    . . . . . C [600]
Lycodes teraoi                     . . . . . A . . . . . C . . . . . T . . . . . G . . . . . . . . . . C [600]
Lycodes toyamaensis                . . . . . A . . . . . C . . . . . T . . . . . G . . . . . G . . . . . [600]
Stichaeus nozawai                  . . . . . A . . . . . C . . A . . C . . . . . T . . . . . G . . T . . . . . [600]
Zoarces americanus                 . . . . . A . . . . . C . . C . . T . . C . . C . . . . . G . . . . . G . . . . . [600]
Zoarces elongatus                  . . . . . A . . . . . C . . C . . . . . C . . C . . . . . T . . . . . A . . . . . G . . . . . . . . . [600]
Zoarces gilli                      . . . . . C . . C . . . . . C . . C . . . . . G . . . . . C [600]

Bothrocara hollandi                A T C C T C T A C C A A C A C C T A T T C T G A T T T [627]
Davidijordania poecillimon         . . . . . T . . . . . T . G . . . . . [627]
Krusensterniella maculata          . . . . . T . . . . . T . G . . . . . [627]
Lycenchelys albomaculata           . . . . . G . . . . . [627]
Lycenchelys aurantiaca             . . . . . T . . . . . G . . . . . [627]
Lycenchelys melanostomias          . C . . . . . G . . . . . [627]
Lycodapus  microchir               . . . . . T . . . . . G . . T . . . . . [627]
Lycodes albonotatus                . . . . . T . . . . . G . . . . . [627]
Lycodes hubbsi                     . . . . . T . . T . . G . . . . . G . . . . . [627]
Lycodes japonicus                  . . . . . T . . . . . G . . . . . [627]
Lycodes matsubarai                 . . . . . T . . . . . G . . . . . [627]
Lycodes nakamurai                  . . . . . T . . . . . G . . . . . [627]
Lycodes ocellatus                  . . . . . T . . . . . G . . . . . G . . . . . [627]
Lycodes pectoralis                 . . . . . T . . . . . G . . . . . [627]
Lycodes raridens                   . . . . . T . . T . . . . . [627]
Lycodes sadoensis                  . . . . . T . . . . . C . . . . . [627]
Lycodes soldatovi                  . . . . . T . . . . . G . . . . . [627]
Lycodes tanakae                    . . . . . T . . T . . . . . T . . G . . . . . [627]
Lycodes teraoi                     . . . . . T . . . . . C . . . . . [627]
Lycodes toyamaensis                . . . . . T . . . . . G . . . . . [627]
Stichaeus nozawai                  . . . . . T . . T . . . . . T . . . . . T . . . . . [627]
Zoarces americanus                 . . . . . T . . . . . T . G . . . . . [627]
Zoarces elongatus                  . . . . . T T . G . . . . . [627]
Zoarces gilli                      . . . . . T . . . . . [627]
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12S rRNA (12S)

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Bothrocara hollandi                A C A C C C A C T A T C C G C C T G G G A A C T A C G A G C A G C A G C T T A A A A C C C A A A G G A C T T G G C G G T [ 60]
Davidijordania poecillimon         . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . . . . . . . . . . . . . . . . [ 60]
Krusensterniella maculata          . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . [ 60]
Lycenchelys albomaculata           . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . [ 60]
Lycenchelys aurantiaca             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycenchelys melanostomias          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodapus  microchir               . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes albonotatus                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . [ 60]
Lycodes hubbsi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes japonicus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes matsubarai                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes nakamurai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes ocellatus                  . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes pectoralis                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes raridens                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes sadoensis                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes soldatovi                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes tanakae                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes teraoi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Lycodes toyamaensis                . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Stichaeus nozawai                  . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . G . . . . . . . . . . . . . . . . . . [ 60]
Zoarces americanus                 . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Zoarces elongatus                  . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . [ 60]
Zoarces gilli                      . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . . . . . . . . . . . . . . . . . . . . [ 60]

Bothrocara hollandi                G C T T T A A A C C C A C C T A G A G G A G C C T G T T C T A G A A C C G A T A C C C C C C G T T C A A C C T C A - C C [120]
Davidijordania poecillimon         . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . [120]
Krusensterniella maculata          . . . . . G . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycenchelys albomaculata           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycenchelys aurantiaca             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycenchelys melanostomias          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodapus  microchir               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes albonotatus                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes hubbsi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes japonicus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes matsubarai                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes nakamurai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes ocellatus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes pectoralis                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes raridens                   . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes sadoensis                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes soldatovi                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes tanakae                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes teraoi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Lycodes toyamaensis                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Stichaeus nozawai                  . . . . . G . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Zoarces americanus                 . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Zoarces elongatus                  . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]
Zoarces gilli                      . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . [120]

Bothrocara hollandi                C T T C C T T G T T T C A C C C G C C T A T A T A C C G C C G T C G T C A G C T T A C C C T G T G A A G G C C T A A T A [180]
Davidijordania poecillimon         T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . [180]
Krusensterniella maculata          . . . . . T . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . [180]
Lycenchelys albomaculata           T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . T T . . . . . [180]
Lycenchelys aurantiaca             T . . . . . C A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . [180]
Lycenchelys melanostomias          T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . A T . . . . . [180]
Lycodapus  microchir               T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . [180]
Lycodes albonotatus                T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes hubbsi                     T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes japonicus                  T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes matsubarai                 T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . G . . . [180]
Lycodes nakamurai                  T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes ocellatus                  T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes pectoralis                 T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes raridens                   T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes sadoensis                  T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes soldatovi                  T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes tanakae                    T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes teraoi                     T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Lycodes toyamaensis                T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . [180]
Stichaeus nozawai                  T . . . . . T C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . [180]
Zoarces americanus                 T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . [180]
Zoarces elongatus                  T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . . . . . [180]
Zoarces gilli                      T . . . . . C T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T T . T . . . [180]

Bothrocara hollandi                G T A A G C A A A A C T G G C C A A G C C C A G A A C G T C A G G T C G A G G T G T A G C G C A T G G G A G G G G A A A G [240]
Davidijordania poecillimon         . . . . . T T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Krusensterniella maculata          . . . . . T . . . . . A . . . . . . . . . . . . . . . . . . . . . T . . . . A G . . . . . . . . . [240]
Lycenchelys albomaculata           . . . . . T T G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycenchelys aurantiaca             . . . . . T . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A G . . . . . . . . . [240]
Lycenchelys melanostomias          . . . . . T . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodapus  microchir               . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes albonotatus                . . . . . T T G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes hubbsi                     . . . . . T T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes japonicus                  . . . . . T T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes matsubarai                 . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . [240]
Lycodes nakamurai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . [240]
Lycodes ocellatus                  . . . . . T T G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes pectoralis                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . [240]
Lycodes raridens                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes sadoensis                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes soldatovi                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes tanakae                    . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes teraoi                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Lycodes toyamaensis                . . . . . T T G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [240]
Stichaeus nozawai                  . . . . . T A . . A . . . C A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . A . . . . . . [240]
Zoarces americanus                 . . . . . T T . . . . . C A . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . [240]
Zoarces elongatus                  . . . . . T T . . . . . T A . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . [240]
Zoarces gilli                      . . . . . T T . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . [240]
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Bothrocara hollandi	A A A T G G G C T A C A T T C G C T G C A G C A G C G A A - T A C G G A C G A T G C A C T G A A A C G T C C A T C T G A	[300]
Davidijordania poecillimon	. . . . C A . . . . - C . . . . T . . . . C . .	[300]
Krusensterniella maculata	. . . . T . . . . C . . . . . . T . . . . T	[300]
Lycenchelys albomaculata	. . . . T . . . . -	[300]
Lycenchelys aurantiaca	. G . . . . A . T . . . . -	[300]
Lycenchelys melanostomias	. . . . C . . . . . A . T . . . . - . . . . . . G	[300]
Lycodapus microchir	. . . . A . . . . - C . . . A	[300]
Lycodes albonotatus	. . . . -	[300]
Lycodes hubbsi	. . . . T . . . . -	[300]
Lycodes japonicus	. . . . T . . . . - . . . . . . T . . . . T	[300]
Lycodes matsubarai	. . . . -	[300]
Lycodes nakamurai	. . . . T A . . . . - . . . . T	[300]
Lycodes ocellatus	. . . . T . . . . - . . . . . . T . . . . T	[300]
Lycodes pectoralis	. . . . T A . . . . -	[300]
Lycodes raridens	. . . . T . . . . -	[300]
Lycodes sadoensis	. . . . A . . . . -	[300]
Lycodes soldatovi	. . . . -	[300]
Lycodes tanakae	. . . . -	[300]
Lycodes teraoi	. . . . A . . . . - C . . . A	[300]
Lycodes toyamaensis	. . . . A . . . . - C	[300]
Stichaeus nozawai	. . . . A . T A . . . . - . . . . T	[300]
Zoarces americanus	. . . . T A . . . . - C . . . . T . . . . C . .	[300]
Zoarces elongatus	. . . . T A . . . . - C . . . . T . . . . C A .	[300]
Zoarces gilli	. . . . - C . . . A . . . . T . . . . C . .	[300]

Bothrocara hollandi	A G G A G G A T T T A G C A G T A A G C A G A A A G T A G C G T G T T C C G C T G A A A T T G G C C C T A A A G C G C G	[360]
Davidijordania poecillimon	. . . . G A . . . A . . . A . . . . C . . . . . . G . . . . . . A	[360]
Krusensterniella maculata	. . . . G . . A . . . A . . . . C . . . . C . . . . G	[360]
Lycenchelys albomaculata	. . . . A . . . A	[360]
Lycenchelys aurantiaca	. . . . A . . . A . . . . G	[360]
Lycenchelys melanostomias	. . . . A . . . A . . . . G . . . . A . .	[360]
Lycodapus microchir	. . . . A	[360]
Lycodes albonotatus	. . . . A . . . A . . . . G	[360]
Lycodes hubbsi	. . . . A . C	[360]
Lycodes japonicus	. . . . A . . . A . . . A . . . . G	[360]
Lycodes matsubarai	. . . . C	[360]
Lycodes nakamurai	. . . . C	[360]
Lycodes ocellatus	. . . . A . . . . G	[360]
Lycodes pectoralis	. . . . C	[360]
Lycodes raridens	. . . . C	[360]
Lycodes sadoensis	. . . . A . . . . G	[360]
Lycodes soldatovi	. . . . C	[360]
Lycodes tanakae	. . . . C	[360]
Lycodes teraoi	. . . . A . . . . G	[360]
Lycodes toyamaensis	. . . . A . . . A . . . . G	[360]
Stichaeus nozawai	. . . . G . . A . . . A . . . . G	[360]
Zoarces americanus	. . . . G . . A . . . A . . . . G	[360]
Zoarces elongatus	. . . . G . . A . . . A . . . . G	[360]
Zoarces gilli	. . . . G . . . . A . C . . . . G	[360]

16S rRNA (16S)

Species	Sequence	Position
Bothrocara hollandi	G C C T G C C C T G T G A C T A T T A G T T T A A C G G C C G C G G T A T T T T G A C C G T G C G A A G G T A G C G C A	[60]
Davidijordania poecillimon	. . . .	[60]
Krusensterniella maculata	. . . .	[60]
Lycenchelys albomaculata	. . . .	[60]
Lycenchelys aurantiaca	. . . .	[60]
Lycenchelys melanostomias	. . . .	[60]
Lycodapus microchir	. . . .	[60]
Lycodes albonotatus	. . . .	[60]
Lycodes hubbsi	. . . .	[60]
Lycodes japonicus	. . . .	[60]
Lycodes matsubarai	. . . .	[60]
Lycodes nakamurai	. . . .	[60]
Lycodes ocellatus	. . . .	[60]
Lycodes pectoralis	. . . .	[60]
Lycodes raridens	. . . .	[60]
Lycodes sadoensis	. . . .	[60]
Lycodes soldatovi	. . . .	[60]
Lycodes tanakae	. . . .	[60]
Lycodes teraoi	. . . .	[60]
Lycodes toyamaensis	. . . .	[60]
Stichaeus nozawai	. . . .	[60]
Zoarces americanus	. . . .	[60]
Zoarces elongatus	. . . .	[60]
Zoarces gilli	. . . .	[60]

Species	Sequence	Position
Bothrocara hollandi	A T C A C T T G T C T T T T A A A T G A A G A C C T G T A T G A A T G G C A T A A C G A G G G C T T A A C T G T C T C C	[120]
Davidijordania poecillimon	. . . .	[120]
Krusensterniella maculata	. . . .	[120]
Lycenchelys albomaculata	. . . .	[120]
Lycenchelys aurantiaca	. . . . G	[120]
Lycenchelys melanostomias	. . . . G	[120]
Lycodapus microchir	. . . .	[120]
Lycodes albonotatus	. . . .	[120]
Lycodes hubbsi	. . . .	[120]
Lycodes japonicus	. . . .	[120]
Lycodes matsubarai	. . . .	[120]
Lycodes nakamurai	. . . .	[120]
Lycodes ocellatus	. . . .	[120]
Lycodes pectoralis	. . . .	[120]
Lycodes raridens	. . . .	[120]
Lycodes sadoensis	. . . .	[120]
Lycodes soldatovi	. . . .	[120]
Lycodes tanakae	. . . . C	[120]
Lycodes teraoi	. . . . C	[120]
Lycodes toyamaensis	. . . .	[120]
Stichaeus nozawai	. . . .	[120]
Zoarces americanus	. . . .	[120]
Zoarces elongatus	. . . .	[120]
Zoarces gilli	. . . .	[120]

Species	Sequence	Position
Bothrocara hollandi	T C T C T C C A G T C A A T G A A A T T G A T C T T C C C G T G C A G A A G C G G G A A T A C A A A C A T A A G A C G A	[180]
Davidijordania poecillimon	. . . . T	[180]
Krusensterniella maculata	. . . .	[180]
Lycenchelys albomaculata	. . . T . . . . G	[180]
Lycenchelys aurantiaca	. T . T . . . . G	[180]
Lycenchelys melanostomias	. T . T . . . . G	[180]
Lycodapus microchir	. T . T . . . . G	[180]
Lycodes albonotatus	. T . T . . . . G	[180]
Lycodes hubbsi	. T . T . . . . G	[180]
Lycodes japonicus	. T . T . . . . G	[180]
Lycodes matsubarai	. T . T . . . . G	[180]
Lycodes nakamurai	. T . T . . . . G	[180]
Lycodes ocellatus	. . . T . . . . G	[180]
Lycodes pectoralis	. . . T . . . . G	[180]
Lycodes raridens	. . . T . . . . G	[180]
Lycodes sadoensis	. . . T . . . . . C . . . . G . . . . T . C	[180]
Lycodes soldatovi	. . . T . . . . G	[180]
Lycodes tanakae	. . . T . . . . . C . . . . G	[180]
Lycodes teraoi	. . . T . . . . . C . . . . G	[180]
Lycodes toyamaensis	. . . T . . . . . C . . . . G	[180]
Stichaeus nozawai	. . . T . . . . . C . . . . G	[180]
Zoarces americanus	. . . T . . . . G	[180]
Zoarces elongatus	. . . T . . . . G	[180]
Zoarces gilli	. . . T . . . . C	[180]

Species	Sequence	Position
Bothrocara hollandi	G A A G A C C C T A T G A A G C T T T A G A C A C C A A G A C A G A T C A T G T T - - T A - A A G G C T T A A A C C A A	[240]
Davidijordania poecillimon	. . . . - - . . -	[240]
Krusensterniella maculata	. . . . A . . . . A A C . -	[240]
Lycenchelys albomaculata	. . . . G . . . . - - C . - . C	[240]
Lycenchelys aurantiaca	. . . . G . . . . . G . C . . . . A A . . - . G	[240]
Lycenchelys melanostomias	. . . . G . C . . . . A A . . - . . . . G	[240]
Lycodapus microchir	. . . . G . . . . A A . . -	[240]
Lycodes albonotatus	. . . . G . . . . A A . . -	[240]
Lycodes hubbsi	. . . . G . . . . A A . . -	[240]
Lycodes japonicus	. . . . G . . . . A A . . -	[240]
Lycodes matsubarai	. . . . G . . . . A A . . -	[240]
Lycodes nakamurai	. . . . G . . . . A A . . -	[240]
Lycodes ocellatus	. . . . G . . . . A A . . - G	[240]
Lycodes pectoralis	. . . . G . . . . A A . . - G	[240]
Lycodes raridens	. . . . G . . . . C . . . . . . A A . . -	[240]
Lycodes sadoensis	. . . . G . . . C . . . . A A . . -	[240]
Lycodes soldatovi	. . . . G . . . . A A . . T . . . A	[240]
Lycodes tanakae	. . . . G . . . . A A C . - . G	[240]
Lycodes teraoi	. . . . G . . . . C . . . . . . A A C . -	[240]
Lycodes toyamaensis	. . . . G . . . . A A . . -	[240]
Stichaeus nozawai	. . . . G . . . . A A . . -	[240]
Zoarces americanus	. . . . G . . . . A A . . - . . . . G C A . . . . . . G	[240]
Zoarces elongatus	. . . . G . . . . A A . . - . . . . G	[240]
Zoarces gilli	. . . . G . . . . G . . . . A A . . - . . . . A	[240]

16S rRNA (16S)

Bothrocara hollandi	A T G A C C T G C C C T A A T G T C T T T G G T T G G G G C G A C C G C G G G G C A T T G A A A A A C C C C C A C G T G	[300]
Davidijordania poecillimon	. . . .	[300]
Krusensterniella maculata	. . . . A	[300]
Lycenchelys albomaculata	G . . . . G	[300]
Lycenchelys aurantiaca	. . . . . . C . . . . G	[300]
Lycenchelys melanostomias	G . . . . . G . . . . G	[300]
Lycodapus microchir	. . . . G	[300]
Lycodes albonotatus	. . . . G	[300]
Lycodes hubbsi	. . . . G	[300]
Lycodes japonicus	. . . . G	[300]
Lycodes matsubarai	. . . G . . . . G	[300]
Lycodes nakamurai	. . . . G	[300]
Lycodes ocellatus	. . . . G	[300]
Lycodes pectoralis	. . . . G	[300]
Lycodes raridens	G . . . . G	[300]
Lycodes sadoensis	G . . . . G	[300]
Lycodes soldatovi	G . . . . G	[300]
Lycodes tanakae	G . . . . G	[300]
Lycodes teraoi	. . . . G	[300]
Lycodes toyamaensis	G . . . . G	[300]
Stichaeus nozawai	. . . . G	[300]
Zoarces americanus	. . . . C . . . G . . . . A . . . . . A . . . A	[300]
Zoarces elongatus	. . . G . . . C . . . . A	[300]
Zoarces gilli	. . . . A	[300]

Bothrocara hollandi	G A G T G G G A G C A T A C A A C T A A G A G C T A C A G C T C T A A T A A A C A G A A A T T C T G A C C A G C A A G A	[360]
Davidijordania poecillimon	. . . . T	[360]
Krusensterniella maculata	. . . . C . . G . . . . A	[360]
Lycenchelys albomaculata	. . A . . . . C . . . . G . . . . A T	[360]
Lycenchelys aurantiaca	. . . . C . . . . G A . . . . A	[360]
Lycenchelys melanostomias	. . C C . . . . C . . . . G . . . . A	[360]
Lycodapus microchir	. . A . . . . G . . . . A	[360]
Lycodes albonotatus	. . A . . . . C . . . . G . . . . A	[360]
Lycodes hubbsi	. . A . . . . C . . . . G . . . . A	[360]
Lycodes japonicus	. . A . . . . C . . . . G	[360]
Lycodes matsubarai	. . A . . . . C . . . . G	[360]
Lycodes nakamurai	. . . . G	[360]
Lycodes ocellatus	. . A . . . . C . . . . G . . . . A T	[360]
Lycodes pectoralis	. . A . . . . C . . . . G . . . . A T	[360]
Lycodes raridens	. . . . C . . . . G . . . . A T	[360]
Lycodes sadoensis	. . A . . . . . . T . . . . C . . . . G . . . . A T	[360]
Lycodes soldatovi	. . A . . . . C . . . . G . . . . A T . G . .	[360]
Lycodes tanakae	. . A . . . . C . . G . . . . . . G . . . G	[360]
Lycodes teraoi	. . A . . . . C . . . . G . . . G	[360]
Lycodes toyamaensis	. . A . . . . C . . . . G	[360]
Stichaeus nozawai	. . A . . . . C . . . . G . . . . T	[360]
Zoarces americanus	. . A . . . . C . . . . . . C . . . . G	[360]
Zoarces elongatus	. . A . . . . C . . . A . . . . . G . . . . C . . T . . . . G . .	[360]
Zoarces gilli	. . A . . . . G . . . . C . . . . G . . . . T	[360]

Bothrocara hollandi	T C C G G C A A T G C C G A T C A A C G G A C C G A G T T A C T C T A G G G A T A A C A G C G C A A T C C T C T T T T A	[420]
Davidijordania poecillimon	. . . .	[420]
Krusensterniella maculata	. . . .	[420]
Lycenchelys albomaculata	. . . .	[420]
Lycenchelys aurantiaca	. . . . C	[420]
Lycenchelys melanostomias	. . . . C	[420]
Lycodapus microchir	. . . . C	[420]
Lycodes albonotatus	. . . . C	[420]
Lycodes hubbsi	. . . . G . . . . C	[420]
Lycodes japonicus	. . . . G . . . . C	[420]
Lycodes matsubarai	. . . . G . . . . C	[420]
Lycodes nakamurai	. . . . G . . . . C	[420]
Lycodes ocellatus	. . . . G . . . . C	[420]
Lycodes pectoralis	. . . . G . . . . C	[420]
Lycodes raridens	. . . . G . . . . C	[420]
Lycodes sadoensis	. . . . G . . . . C	[420]
Lycodes soldatovi	. . . . A . . . . . . C	[420]
Lycodes tanakae	. . . . C	[420]
Lycodes teraoi	. . . . C	[420]
Lycodes toyamaensis	. . . . C	[420]
Stichaeus nozawai	. . . . C	[420]
Zoarces americanus	. . . . A . . . . C	[420]
Zoarces elongatus	. . . . C	[420]
Zoarces gilli	. . . . C	[420]

Bothrocara hollandi	G A G C C C A T A T C G A C A A G G G G G T T T A C G A C C T C G A T G T T G G A T C A G G A C A T C C T A A T G G T G	[480]
Davidijordania poecillimon	. . . .	[480]
Krusensterniella maculata	. . . .	[480]
Lycenchelys albomaculata	. . . . G . . C	[480]
Lycenchelys aurantiaca	. . . .	[480]
Lycenchelys melanostomias	. . . .	[480]
Lycodapus microchir	. . . .	[480]
Lycodes albonotatus	. . . .	[480]
Lycodes hubbsi	. . . .	[480]
Lycodes japonicus	. . . .	[480]
Lycodes matsubarai	. . . .	[480]
Lycodes nakamurai	. . . .	[480]
Lycodes ocellatus	. . . .	[480]
Lycodes pectoralis	. . . .	[480]
Lycodes raridens	. . . .	[480]
Lycodes sadoensis	. . . .	[480]
Lycodes soldatovi	. . . .	[480]
Lycodes tanakae	. . . .	[480]
Lycodes teraoi	. . . .	[480]
Lycodes toyamaensis	. . . .	[480]
Stichaeus nozawai	. . . .	[480]
Zoarces americanus	. . . .	[480]
Zoarces elongatus	. . . .	[480]
Zoarces gilli	. . . .	[480]

16S rRNA (16S)

Bothrocara hollandi	C A G C C G C T A T T A A G G G T T C G T T T G T T C A A C G A T T A A A G T C C T	[522]
Davidijordania poecillimon	. . . . .	[522]
Krusensterniella maculata	. . . . .	[522]
Lycenchelys albomaculata	. . . . .	[522]
Lycenchelys aurantiaca	. . . . .	[522]
Lycenchelys melanostomias	. . . . .	[522]
Lycodapus microchir	. . . . .	[522]
Lycodes albonotatus	. . . . .	[522]
Lycodes hubbsi	. . . . .	[522]
Lycodes japonicus	. . . . .	[522]
Lycodes matsubarai	. . . . .	[522]
Lycodes nakamurai	. . . . .	[522]
Lycodes ocellatus	. . . . .	[522]
Lycodes pectoralis	. . . . .	[522]
Lycodes raridens	. . . . .	[522]
Lycodes sadoensis	. . . . .	[522]
Lycodes soldatovi	. . . . .	[522]
Lycodes tanakae	. . . . .	[522]
Lycodes teraoi	. . . . .	[522]
Lycodes toyamaensis	. . . . .	[522]
Stichaeus nozawai	. . . . .	[522]
Zoarces americanus	. . . . .	[522]
Zoarces elongatus	. . . . .	[522]
Zoarces gilli	. . . . .	[522]

Intron region of nuclear Rhodopsin ggene (Rhodopsin)

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Bothrocara hollandi          G G T G C C T A C A T G T T T C T G C T C A T C C T C G T A G G C T T C C C C G T C A A C T C C C T C A C C C T G T A C [ 60]
Davidijordania poecillimon   . . . . . T . . . . . [ 60]
Krusensterniella maculata    . . . . . T . . . . . [ 60]
Lycenchelys albomaculata     . . . . . [ 60]
Lycenchelys aurantiaca       . . . . . [ 60]
Lycenchelys melanostomias    . . . . . [ 60]
Lycodapus  microchir         . . . . . G . . . . . [ 60]
Lycodes albonotatus          . . . . . [ 60]
Lycodes hubbsi               . . . . . T . . . . . A . . . . . [ 60]
Lycodes japonicus            . . . . . A . . . . . [ 60]
Lycodes matsubarai           . . . . . [ 60]
Lycodes nakamurai            . . C . . . . . G . . . . . [ 60]
Lycodes ocellatus            . . . . . T . . . . . A . . . . . [ 60]
Lycodes pectoralis           . . C . . . . . [ 60]
Lycodes raridens             . . . . . [ 60]
Lycodes sadoensis            . . . . . [ 60]
Lycodes soldatovi            . . . . . T . . . . . C . . . . . A . . . . . [ 60]
Lycodes tanakae              . . . . . [ 60]
Lycodes teraoi               . . . . . [ 60]
Lycodes toyamaensis          . . . . . [ 60]
Stichaeus nozawai            . . . . . T . . . . . [ 60]
Zoarces americanus           . . . . . T . . . . . [ 60]
Zoarces elongatus            . . . . . T . . . . . T . . . . . [ 60]
Zoarces gilli                . . . . . A . . . . . T . . . . . [ 60]

Bothrocara hollandi          G T C A C C C T C G A A C A C A A G A A G C T G C G G A C C C C T C T A A A C T A C A T C C T G C T G A A C C T G G C G [120]
Davidijordania poecillimon   . . . . . [120]
Krusensterniella maculata    . . . . . [120]
Lycenchelys albomaculata     . . . . . [120]
Lycenchelys aurantiaca       . . . . . [120]
Lycenchelys melanostomias    . . . . . [120]
Lycodapus  microchir         . . . . . C . . . . . [120]
Lycodes albonotatus          . . . . . [120]
Lycodes hubbsi               . . . . . T . . . . . [120]
Lycodes japonicus            . . . . . T . . . . . [120]
Lycodes matsubarai           . . . . . [120]
Lycodes nakamurai            . . . . . C . . . . . [120]
Lycodes ocellatus            . . . . . T . . . . . [120]
Lycodes pectoralis           . . . . . C . . . . . [120]
Lycodes raridens             . . . . . [120]
Lycodes sadoensis            . . . . . [120]
Lycodes soldatovi            . . . . . [120]
Lycodes tanakae              . . . . . [120]
Lycodes teraoi               . . . . . [120]
Lycodes toyamaensis          . . . . . [120]
Stichaeus nozawai            . . . . . C . . . . . [120]
Zoarces americanus           . . . . . [120]
Zoarces elongatus            . . . . . [120]
Zoarces gilli                . . . . . [120]

Bothrocara hollandi          G T G G C C A A C C T C T T C A T G G T G C T G G G A G G G T T C A C C A C G A C G A T G T A C A C C T C C A T G C A C [180]
Davidijordania poecillimon   . . . . . [180]
Krusensterniella maculata    . . . . . C . . . . . [180]
Lycenchelys albomaculata     . . . . . [180]
Lycenchelys aurantiaca       . . . . . [180]
Lycenchelys melanostomias    . . . . . [180]
Lycodapus  microchir         . . . . . [180]
Lycodes albonotatus          . . . . . [180]
Lycodes hubbsi               . . . . . [180]
Lycodes japonicus            . . . . . [180]
Lycodes matsubarai           . . . . . [180]
Lycodes nakamurai            . . . . . [180]
Lycodes ocellatus            . . . . . [180]
Lycodes pectoralis           . . . . . A . . . . . [180]
Lycodes raridens             . . . . . [180]
Lycodes sadoensis            . . . . . [180]
Lycodes soldatovi            . . . . . [180]
Lycodes tanakae              . . . . . [180]
Lycodes teraoi               . . T . . . . . [180]
Lycodes toyamaensis          . . . . . [180]
Stichaeus nozawai            . . . . . [180]
Zoarces americanus           . . . . . [180]
Zoarces elongatus            . . . . . G . . . . . [180]
Zoarces gilli                . . . . . [180]

Bothrocara hollandi          G G C T A C T T C G T G C T G G G T C G C C T C G G C T G C A A C C T G G A A G G A T T C T T T G C A A C C C T G G G C [240]
Davidijordania poecillimon   . . . . . A . . . . . G . . . . . A . . . . A [240]
Krusensterniella maculata    . . . . . G . . . . . A . . . . . [240]
Lycenchelys albomaculata     . . . . . [240]
Lycenchelys aurantiaca       . . . . . [240]
Lycenchelys melanostomias    . . . . . [240]
Lycodapus  microchir         . . . . . G . . . . . [240]
Lycodes albonotatus          . . . . . G . . . . . [240]
Lycodes hubbsi               . . . . . G . . . . . [240]
Lycodes japonicus            . . . . . G . . . . . [240]
Lycodes matsubarai           . . . . . [240]
Lycodes nakamurai            . . . . . [240]
Lycodes ocellatus            . . . . . G . . . . . [240]
Lycodes pectoralis           . . . . . A . . . . . [240]
Lycodes raridens             . . . . . [240]
Lycodes sadoensis            . . . . . [240]
Lycodes soldatovi            . . . . . G . . . . . [240]
Lycodes tanakae              . . . . . [240]
Lycodes teraoi               . . . . . [240]
Lycodes toyamaensis          . . . . . G . . . . . [240]
Stichaeus nozawai            . . . . . A . . . . . G . . . . . A [240]
Zoarces americanus           . . . . . T . . . . . [240]
Zoarces elongatus            . . . . . G . . . . . [240]
Zoarces gilli                . . . . . G . . . . . G . . . . . T . . . . . [240]
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Intron region of nuclear Rhodopsin ggene (Rhodopsin)

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Bothrocara hollandi                 G G T G A G A T A G C C C T C T G G T C G C T G G T C G T T C T G G C T A T C G A G A G G T G G G T G G T C G T C T G C [300]
Davidijordania poecillimon          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Krusensterniella maculata           . . . . . . . . C . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . [300]
Lycenchelys albomaculata            . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycenchelys aurantiaca              . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycenchelys melanostomias           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodapus  microchir                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes albonotatus                 . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes hubbsi                      . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes japonicus                   . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes matsubarai                  . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes nakamurai                   . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes ocellatus                   . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes pectoralis                  . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes raridens                    . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes sadoensis                   . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes soldatovi                   . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . [300]
Lycodes tanakae                     . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes teraoi                      . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Lycodes toyamaensis                 . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Stichaeus nozawai                   . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Zoarces americanus                  . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Zoarces elongatus                   . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]
Zoarces gilli                       . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [300]

Bothrocara hollandi                 A A G C C C A T C G C C A A C T T C C G C T T C A G C G A G G A T C A C G C T A T C A T G G G T C T G G C C T T C A C C [360]
Davidijordania poecillimon          . . . . . . . . . . A G . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . [360]
Krusensterniella maculata           . . . . . . . . . . A G . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . [360]
Lycenchelys albomaculata            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycenchelys aurantiaca              . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycenchelys melanostomias           . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodapus  microchir                . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes albonotatus                 . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . T . . . . . . . . . . . . G . . . . . [360]
Lycodes hubbsi                      . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . [360]
Lycodes japonicus                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . [360]
Lycodes matsubarai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes nakamurai                   . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . [360]
Lycodes ocellatus                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . [360]
Lycodes pectoralis                  . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . [360]
Lycodes raridens                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes sadoensis                   . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . A . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes soldatovi                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes tanakae                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes teraoi                      . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . A . . . . . . . . . . . . . . . . . . . . . . . [360]
Lycodes toyamaensis                 . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . G . . . A . . . [360]
Stichaeus nozawai                   . . . . . . . . . . A G . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . T [360]
Zoarces americanus                  . . . . . . . . . . A G . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . [360]
Zoarces elongatus                   . . . . . . . . . . A G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . [360]
Zoarces gilli                       . . . . . . . . . . A G . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . [360]

Bothrocara hollandi                 T G G A C C A T G G C C T T G G C T T G C T C C G T G C C C C C T C T C G T C G G C T G G T C T C G T T A C A T C C C C [420]
Davidijordania poecillimon          . . . G T . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Krusensterniella maculata           . . . G T . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycenchelys albomaculata            . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycenchelys aurantiaca              . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycenchelys melanostomias           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodapus  microchir                . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes albonotatus                 . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes hubbsi                      . . . . . . . . . . . . . A . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes japonicus                   . . . . . . . . . . . . . A . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes matsubarai                  . . . . . . . . . . . . . . C . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes nakamurai                   . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes ocellatus                   . . . . . . . . . . . . . A . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes pectoralis                  . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes raridens                    . . . G . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G [420]
Lycodes sadoensis                   . . . G . . . . . . . . . C . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes soldatovi                   . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes tanakae                     . . . G T . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes teraoi                      . . . G . . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Lycodes toyamaensis                 . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Stichaeus nozawai                   . . . G T . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Zoarces americanus                  . . . G T . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Zoarces elongatus                   . . . G T . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]
Zoarces gilli                       . . . G T . . . . . C . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [420]

Bothrocara hollandi                 G A G G G C A T G C A G T G C T C A T G C G G A A T C G A C T A C T A C A C G C G T G C G G A G G G T T T C A A C A A C [480]
Davidijordania poecillimon          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Krusensterniella maculata           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycenchelys albomaculata            . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycenchelys aurantiaca              . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycenchelys melanostomias           . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodapus  microchir                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes albonotatus                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes hubbsi                      . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes japonicus                   . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes matsubarai                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes nakamurai                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes ocellatus                   . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes pectoralis                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes raridens                    . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes sadoensis                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes soldatovi                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes tanakae                     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes teraoi                      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Lycodes toyamaensis                 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Stichaeus nozawai                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Zoarces americanus                  . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Zoarces elongatus                   . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
Zoarces gilli                       . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . [480]
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Intron region of nuclear Rhodopsin ggene (Rhodopsin)

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Bothrocara hollandi                G A G T C C T T C G T G C T C T A C A T G T T C A G C T G C C A C T T C C T C G C C C C G C T G A C T G T C A T T T T C [540]
Davidijordania poecillimon         . . . . . T G . . . . . C . . . . . A . . . . . G T . . . . . [540]
Krusensterniella maculata          . . . . . T A . . . . . C . . . . . A T . . . . . G T . . . . . [540]
Lycenchelys albomaculata           . . . . . T A . . . . . C . . . . . A . . . . . [540]
Lycenchelys aurantiaca             . . . . . T A . . . . . C . . . . . A . . . . . [540]
Lycenchelys melanostomias          . . . . . T A . . . . . C . . . . . A . . . . . [540]
Lycodapus microchir                . . . . . T A . . . . . A . . . . . [540]
Lycodes albonotatus                . . . . . T A . . . . . C . . . . . A . . . . . [540]
Lycodes hubbsi                     . . . . . T G . . . . . C . . . . . A . . . . . A . . . . . [540]
Lycodes japonicus                  . . . . . T . . . . . C . . . . . A . . . . . A . . . . . [540]
Lycodes matsubarai                 . . . . . T A . . . . . A . . . . . [540]
Lycodes nakamurai                  . . . . . T A . . . . . A . . . . . [540]
Lycodes ocellatus                  . . . . . T . . . . . C . . . . . A . . . . . A . . . . . [540]
Lycodes pectoralis                 . . . . . T A . . . . . A . . . . . [540]
Lycodes raridens                   . . . . . T A . . . . . A . . . . . [540]
Lycodes sadoensis                  . . . . . T A . . . . . C . . . . . A . . . . . [540]
Lycodes soldatovi                  . . . . . T A . . . . . A . . . . . [540]
Lycodes tanakae                    . . . . . T A . . . . . A . . . . . [540]
Lycodes teraoi                     . . . . . T A . . . . . C . . . . . A . . . . . [540]
Lycodes toyamaensis                . . . . . T A . . . . . C . . . . . A . . . . . [540]
Stichaeus nozawai                  . . . . . T A . . . . . C . . . . . A A . . . . . T . A . . . . . [540]
Zoarces americanus                 . . . . . T A . . . . . T . . . . . A T . . . . A . . G T . . . . . [540]
Zoarces elongatus                  . . . . . T A . . . . . C . . . . . G . . A T . . . . A . . G T . . . . . [540]
Zoarces gilli                      . . . A . . . . . T A . . . . . C . . . . . A T . . . . A . . G T . . . . . [540]

Bothrocara hollandi                T T C T G C T A C G G A C G C C T G C T C T G C G C C G T C A A G G A C G C C G C C G C C G C C C A G C A G G A G T C C [600]
Davidijordania poecillimon         . . . . . G . . . . . G . . . . . [600]
Krusensterniella maculata          . . . . . G . . . . . [600]
Lycenchelys albomaculata           . . . . . G . . . . . [600]
Lycenchelys aurantiaca             . . . . . G . . . . . [600]
Lycenchelys melanostomias          . . . . . G . . . . . [600]
Lycodapus microchir                . . . . . G . . . . . [600]
Lycodes albonotatus                . . . . . G . . . . . [600]
Lycodes hubbsi                     . . . . . G . . . . . [600]
Lycodes japonicus                  . . . . . G . . . . . [600]
Lycodes matsubarai                 . . . . . G . . . . . [600]
Lycodes nakamurai                  . . . . . G . . . . . [600]
Lycodes ocellatus                  . . . . . G . . . . . [600]
Lycodes pectoralis                 . . . . . G . . . . . [600]
Lycodes raridens                   . . . . . G . . . . . [600]
Lycodes sadoensis                  . . . . . G . . . . . [600]
Lycodes soldatovi                  . . . . . G . . . . . [600]
Lycodes tanakae                    . . . . . A . . . . . G . . . . . [600]
Lycodes teraoi                     . . . . . G . . . . . [600]
Lycodes toyamaensis                . . . . . G . . . . . [600]
Stichaeus nozawai                  . . . . . G . . . . . [600]
Zoarces americanus                 . . . . . G . . . . . [600]
Zoarces elongatus                  . . . . . G . . . . . [600]
Zoarces gilli                      . . . . . A . . . . . G . . . . . [600]

Bothrocara hollandi                G A G A C C A C C C A G A G G G C C G A G A G G G A G G T C A G C C G C A T G G T G G T G A T C A T G G T C A T C G C C [660]
Davidijordania poecillimon         . . . . . [660]
Krusensterniella maculata          . . . . . [660]
Lycenchelys albomaculata           . . . . . [660]
Lycenchelys aurantiaca             . . . . . [660]
Lycenchelys melanostomias          . . . . . [660]
Lycodapus microchir                . . . . . [660]
Lycodes albonotatus                . . . . . T . . . . . [660]
Lycodes hubbsi                     . . . . . [660]
Lycodes japonicus                  . . . . . [660]
Lycodes matsubarai                 . . . . . [660]
Lycodes nakamurai                  . . . . . [660]
Lycodes ocellatus                  . . . . . [660]
Lycodes pectoralis                 . . . . . [660]
Lycodes raridens                   . . . . . [660]
Lycodes sadoensis                  . . . . . [660]
Lycodes soldatovi                  . . . . . [660]
Lycodes tanakae                    . . . . . [660]
Lycodes teraoi                     . . . . . [660]
Lycodes toyamaensis                . . . . . T . . . . . [660]
Stichaeus nozawai                  . . . . . G . . . . . [660]
Zoarces americanus                 . . . . . A . . . . . [660]
Zoarces elongatus                  . . . . . A . . . . . [660]
Zoarces gilli                      . . . . . A . . . . . [660]

Bothrocara hollandi                T T C C T G G T G T G T T G G G T G C C C T A C G C C A G C G T G G C C C T G G T T T A T C T T C T G T A A C C A G G G A [720]
Davidijordania poecillimon         . . . . . A C . . . . . C . . . . . [720]
Krusensterniella maculata          . . . . . G G . . . . . [720]
Lycenchelys albomaculata           . . . . . [720]
Lycenchelys aurantiaca             . . . . . [720]
Lycenchelys melanostomias          . . . . . [720]
Lycodapus microchir                . . . . . T . . . . . [720]
Lycodes albonotatus                . . . . . [720]
Lycodes hubbsi                     . . . . . [720]
Lycodes japonicus                  . . . . . [720]
Lycodes matsubarai                 . . . . . [720]
Lycodes nakamurai                  . . . . . [720]
Lycodes ocellatus                  . . . . . [720]
Lycodes pectoralis                 . . . . . [720]
Lycodes raridens                   . . . . . [720]
Lycodes sadoensis                  . . . . . [720]
Lycodes soldatovi                  . . . . . [720]
Lycodes tanakae                    . . . . . [720]
Lycodes teraoi                     . . . . . [720]
Lycodes toyamaensis                . . . . . [720]
Stichaeus nozawai                  . . . . . [720]
Zoarces americanus                 . . . . . G G . . . . . [720]
Zoarces elongatus                  . A . . . . . G G . . . . . [720]
Zoarces gilli                      . . . A . T . . . . . G G . . . . . [720]
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Intron region of nuclear Rhodopsin ggene (Rhodopsin)

Bothrocara hollandi	T C C G A G T T C G G A C C G G T C T T C A T G A C C A T C C C G T C C T T C	[759]
Davidijordania poecillimon	. . . . . G	[759]
Krusensterniella maculata	. . . . . C . . . . . G	[759]
Lycenchelys albomaculata	. . . . .	[759]
Lycenchelys aurantiaca	. . . . .	[759]
Lycenchelys melanostomias	. . T	[759]
Lycodapus microchir	. . . . . G	[759]
Lycodes albonotatus	. . . . . G	[759]
Lycodes hubbsi	. . . . . G	[759]
Lycodes japonicus	. . . . . G	[759]
Lycodes matsubarai	. . . . . A . . . . . G	[759]
Lycodes nakamurai	. . . . . G	[759]
Lycodes ocellatus	. . . . . G	[759]
Lycodes pectoralis	. . . . . G	[759]
Lycodes raridens	. . . . . G	[759]
Lycodes sadoensis	. . . . . G	[759]
Lycodes soldatovi	. . . . . G	[759]
Lycodes tanakae	. . . . . G	[759]
Lycodes teraoi	. . . . . G	[759]
Lycodes toyamaensis	. . . . . G . . . C T	[759]
Stichaeus nozawai	. . . . . C . . . . . G	[759]
Zoarces americanus	. . . . . C . . . . . G	[759]
Zoarces elongatus	. . . . . G	[759]
Zoarces gilli	. . . . . G	[759]