Origin and intraspecific diversification of the scincid lizard *Ateuchosaurus pellopleurus* with implications for historical island biogeography of the Central Ryukyus of Japan

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ABSTRACT

The Central Ryukyus, a group of islands in southwestern Japan, harbor many endemic reptiles and amphibians, and the geographic ranges of the endemics are limited to south of the Tokara Gap, an old sea strait. *Ateuchosaurus pellopleurus* is a lizard with an exceptionally wide geographic range that encompasses the Central Ryukyus and north of the Tokara Gap. The intraspecific geographic variation of this species and the divergence time between *A. pellopleurus* and its sister species in southern China, *A. chinensis*, were assessed by molecular phylogeographic analyses. The results indicated that the populations north of the gap were established by recent northward dispersal, prior to which the distribution of this species was limited to the Central Ryukyus. The estimated divergence time between *A. pellopleurus* and *A. chinensis* was in the Oligocene or Miocene, which was concordant with the divergence times of most Central Ryukyu endemics. These results demonstrated that *A. pellopleurus* is essentially a Central Ryukyu endemic that shares a common biogeographic history with other endemics. The common divergence times suggested that isolation of the endemics from their sister species in surrounding areas are attributable to geographic isolation by a tectonic event in the Miocene. In addition, *A. pellopleurus* showed distinct divergence between the populations in the northern and southern parts of the Central Ryukyus, and further diversification within and between islands in the southern part. Comparison of the phylogeographic patterns between this species and other endemics revealed that the present diversity in the Central Ryukyu endemics was formed by a complex history that involved occasional dispersal rather than simple vicariance events.

Keywords:

Dispersal; Divergence time; Phylogeography; Population genetics; Scincidae; Central Ryukyus

1. Introduction

In traditional historical biogeography, common patterns in geographic distributions and sister relationships that are shared by co-occurring taxa were considered evidence of past vicariance events (Crisci et al. 2003; Wiley & Lieberman 2011). Recent progresses of molecular biogeographic studies have highlighted that congruent divergence times among taxa could also reveal common vicariance events (Donoghue & Moore 2003). In addition, population genetic studies have revealed important biogeographic insights, such as recent founder events of local populations (Excoffier & Ray 2008; Excoffier et al. 2009). Thus, various molecular approaches provide opportunities to address detailed questions in modern historical biogeography.

The Ryukyu Archipelago, of which the historical biogeography has been extensively investigated, is located between the Japanese main islands and Taiwan in East Asia, and can be divided into several groups (split herein into Osumi, Tokara, Amami, Okinawa, and Sakishima Groups, Fig. 1). Previous biogeographic reviews of terrestrial reptiles and amphibians that inhabit the archipelago (Hikida & Ota 1997; Ota 1998, Ota 2000) revealed strong endemicity in the central part of the archipelago, which consists of the southern part of the Tokara Group (“southern Tokara” hereafter; sites 8 and 9 in Fig. 1) to the Okinawa Group (“the Central Ryukyus” hereafter; see Kaito & Toda 2016; Okamoto 2017); alternatively, the Osumi Group (sites 1–3 and adjacent islands in Fig. 1) and the northern part of the Tokara Group (“northern Tokara” hereafter; sites 4–7 in Fig. 1) harbored species that also inhabit the

Japanese main islands, and species distributed in the Sakishima Group were related to Taiwanese and/or southern continental Chinese taxa. The northern and southern limits of most Central Ryukyu endemics are located along the sea straits, which correspond to the major tectonic depressions, the Tokara and Kerama Gaps, respectively (Fig. 1).

Based on prior molecular phylogenetic studies on Ryukyu terrestrial reptiles (e.g., Toda et al. 1999; Ota et al. 2002; Lue & Lin 2008), a recent review of Japanese terrestrial reptile historical biogeography (Okamoto 2017) mentioned that most of the Central Ryukyu endemics had their closest relatives in surrounding areas (i.e., the Japanese main islands, the Sakishima Group, Taiwan, and/or the Asian continent), with divergence times around the late Miocene. The review also suggested that some endemics were relicts with exceptionally older origins around the Eocene, whereas others might have colonized the area by overseas dispersal, with younger origins in the Pliocene (Okamoto 2017).

Several exceptions that widely occur in the Central Ryukyus and surrounding areas have also been identified, and their variable biogeographic backgrounds were clarified by molecular studies. A rhacophorid frog, *Buergeria japonica* (Hallowell, 1861), which is distributed from the Ryukyu Archipelago to Taiwan, was revealed to have several diverged lineages; one lineage occurs throughout the Central Ryukyus and northern Tokara, which indicates recent northward overseas dispersal through the Tokara Gap (Tominaga et al. 2015). The biogeographic history of *B. japonica* was thus deemed to be congruent with the general biogeographic patterns of the Central Ryukyu endemics. By contrast, previous studies also revealed truly incongruent patterns in *Gekko hokouensis* Pope, 1928 (Toda et al. 1997a) and complex genetic variations with the mito-nuclear discordance in *Fejervarya kawamurai* Djong, Matsui, Kuramoto, Nishioka & Sumida, 2011 (Toda et al. 1997b; Sumida et al. 2007), compared with the general patterns of endemics. Therefore, molecular phylogeographic studies can help clarify whether species may be essentially congruent or truly incongruent

with general biogeographic patterns.

Ateuchosaurus pellopleurus (Hallowell, 1861) is a scincid lizard that occurs throughout the Central Ryukyus, northern Tokara, and part of the Osumi Group (Fig. 1B). Its only congener, *Ateuchosaurus chinensis* Gray, 1845, is indigenous to southern continental China and northeastern Vietnam (Fig. 1A), although a sister relationship of these species has never been assessed by molecular analyses. Based on the distant occurrence of its congener, *A. pellopleurus* was sometimes considered a biogeographic relict (e.g., Hikida & Ota 1997). Okamoto (2017) also speculated that *A. pellopleurus* could be an old relict among the Central Ryukyu endemics, rather than the majority of Central Ryukyu endemics, which have a late Miocene origin. To clarify the biogeographic history of *A. pellopleurus*, the monophyly and divergence time of *Ateuchosaurus* Gray, 1845 should be examined by molecular approaches. This needs a phylogenetic analysis of *Ateuchosaurus* species and a wide range of other scincid lizards given the inconsistent phylogenetic results in previous studies (Austin & Arnold 2006; Pyron et al. 2013; Zheng & Wiens 2016). In addition to the disjunct distribution with its congener, *A. pellopleurus* exhibits a deviation from the general biogeographic patterns of most other endemics, because this species occurs in both northern and southern sides of the Tokara Gap (Hikida et al. 1992).

A morphological study based on morphometric and scale characters of *A. pellopleurus* revealed little differentiation between the northern and southern sides of the gap, which indicated that the population on the northern side was established by overseas dispersal through the Tokara Gap (Ota et al. 1999). Because various diversification patterns along the Tokara Gap were suggested in terrestrial amphibians and reptiles (Toda et al. 1997a, 1997b; Tominaga et al. 2015), recent range expansions in *A. pellopleurus* should be corroborated by population genetic analyses. Although Ota et al. (1999) also revealed gradual geographic variation within *A. pellopleurus* populations that inhabit the Central Ryukyus, the pattern

contrasted the general patterns observed in most co-occurring species in the Central Ryukyus; they showed distinct genetic divergences between the populations of the Okinawa and Amami Groups (e.g., Tominaga et al. 2015; Kaito & Toda 2016; Kaito et al. 2017; Tominaga et al. 2019). Because the gradual variation of *A. pellopleurus* was determined by a few morphological characters (Ota et al. 1999), this variation in the Central Ryukyus should also be confirmed by molecular assessment. If *A. pellopleurus* has the gradual geographic variation along the Central Ryukyus, discordant geographic boundaries among different loci or intermediate allele composition in geographically intermediate populations would be expected.

In this study, we addressed the following three biogeographic issues: 1) whether *A. pellopleurus* deviates from the general biogeographic patterns of Central Ryukyu endemics; 2) whether this species is a relict with an old origin or an ordinary Central Ryukyu endemic with a Miocene origin; and 3) whether this species exhibits gradual geographic variation within the Central Ryukyus. The first issue was tested by examining the population genetic characteristics of the populations around the Tokara Gap. The second issue was addressed by time-calibrated molecular phylogenetic assessment of the monophyly of *Ateuchosaurus* and the isolation time of *A. pellopleurus*. Finally, the third issue was addressed by examining a concordance between differentiation patterns of mitochondrial and nuclear DNA loci along the Central Ryukyus. Accordingly, we provide further insight into the origin and historical biogeography of *A. pellopleurus* and the Central Ryukyu endemic terrestrial reptiles.

2. Material and methods

2.1. Sampling

In total, 167 *A. pellopleurus* specimens were collected from 37 localities on 27 islands, which covered the known distribution of this skink (Fig 1; Appendix S1). We also examined four specimens of *A. chinensis* from three localities. The specimens were deposited in the Zoological Collection of Kyoto University (KUZ; see Appendix S1 for details). In addition, homologous DNA sequences of 38 squamate species, including 27 scincid lizards, were obtained from GenBank (Appendix S2) and used as outgroups for phylogenetic analyses.

2.2. DNA sequencing

We prepared two DNA sequence datasets for time-calibrated molecular phylogenies. One was a concatenated dataset of seven nuclear DNA fragments (*BDNF*, *MKL1*, *PRLR*, *PTGER4*, *R35*, *RAG1*, and *SNCAIP*) of eight *A. pellopleurus* specimens that represented the two major northern and southern lineages recognized in a preliminary analysis (not shown), four *A. chinensis*, and other outgroup taxa (Appendix S2). The other dataset consisted of a mitochondrial DNA (mtDNA) cytochrome *b* fragment of 161 *A. pellopleurus* specimens and one *A. chinensis* specimen. In addition, *RAG1* sequences were also determined for 72 *A. pellopleurus* specimens to cover all of the recognized intraspecific mtDNA clades (Appendix S1).

Genomic DNA was extracted from liver or muscle tissue following the method described by Okamoto et al. (2006). Polymerase chain reaction (PCR) was conducted to amplify partial fragments of cytochrome *b* and the seven nuclear gene fragments using a *Takara Ex Taq* kit (Takara Bio, Kusatsu, Japan) with Gene Amp PCR System 2700 or 9700 (Thermo Fisher Scientific, Waltham, MA, USA). The primers and PCR conditions for amplifications followed those described by Okamoto & Hikida (2009) for cytochrome *b*, Kurita & Hikida (2014a) for

RAG1, and Brandley et al. (2011) for the other nuclear DNA fragments. The PCR products were purified as described by Okamoto & Hikida (2009).

The purified PCR products were used for sequencing. Nucleotide sequencing was conducted for both strands of each PCR product using a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) with the sequencing primers used by Okamoto & Hikida (2009), Kurita & Hikida (2014a), and Brandley et al. (2011), and a newly designed primer (L-749RAG1At: 5′-AAC CTA GAG CGG TAT GAG ATG-3′) for *RAG1*. Sequencing was then performed using an Applied Biosystems 3130*xl* Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) or the commercial sequencing service of Macrogen Japan Corp., which also used an Applied Biosystems 3730*xl* DNA Analyzer. The resultant sequences of complementary strands for each fragment were assembled using Gap4 (Staden et al. 2003). After checking for absences of stop codons and insertions/deletions by translating DNA sequences into amino acid residual sequences using MEGA6 (Tamura et al. 2013) or MEGA7 (Kumar et al. 2016), the sequences were aligned by eye.

2.3. Phylogenetic reconstruction

There are no known *Ateuchosaurus* fossils appropriate for calibration of divergence times within and between species of this genus. Therefore, the divergence times between the two *Ateuchosaurus* species and among major lineages of *A. pellopleurus* were first estimated using the seven nuclear DNA dataset by calibrating the outgroup tree. Then, a time-calibrated intraspecific phylogeny of *A. pellopleurus* was inferred using the cytochrome *b* dataset with secondary calibration based on the resultant estimates of the first analysis. We employed Bayesian inference (BI) to reconstruct a time-calibrated tree using BEAST v2.5.0 (Bouckaert

et al. 2019). In the outgroup tree inference, the dataset was split into *a priori* partitions of 1st, 2nd, and 3rd triplet positions of each locus, and the partitioning regime and substitution model of each partition (Appendix S3) were selected by PartitionFinder2 (Lanfear et al. 2016) under the Bayesian information criterion (Schwarz 1978). We used the Yule prior, an uncorrelated lognormal relaxed molecular clock, and a uniform prior of 0–100 for the shape parameter of gamma distribution of rate heterogeneity of each partition. For the other parameters, default priors were used. For divergence time calibration, we employed six constraints based on the preceding studies and fossil records described in Appendix S4.

Selection of a partitioned model for the ingroup phylogeny reconstruction was performed by PartitionFinder2 based on *a priori* partitions of the 1st, 2nd, and 3rd triplet positions of the cytochrome *b* sequences (Appendix S3). The time-calibrated ingroup phylogeny was inferred by BI based on a non-collapsed dataset of all sequenced specimens. In the BI, we used the coalescent constant population model tree prior and a strict molecular clock. We also used a uniform prior of 0–1 for the shape parameter of gamma distribution of rate heterogeneity of each partition. For the other parameters, default priors were used. For both of the outgroup and ingroup analyses, we conducted two independent Markov chain Monte Carlo (MCMC) analyses with 100,000,000 iterations and sampled every 50,000 states, and the initial 10% were discarded as burn-in. Then, effective sample size for each parameter of each MCMC run and congruence of each parameter between the independent MCMC analyses were checked by Tracer v1.6 (Rambaut et al. 2013).

The *RAG1* haplotypes were inferred using PHASE v2.1 (Stephens et al. 2001; Stephens & Donnelly 2003) implemented in DnaSP v5.10 (Librado & Rozas 2009). PHASE analysis was separately performed for samples from the Okinawa Group and other areas under default settings. The results with ≥ 0.9 posterior probabilities were accepted. Then, the *RAG1* haplotype relationships were inferred by statistical parsimony (Templeton et al. 1992) using

TCS v1.21 (Clement et al. 2000).

2.4. Test of recent population expansion around the Tokara Gap

To test the hypothesis of recent dispersal across the Tokara Gap postulated by Ota et al. (1999), we determined if there was population expansion based on the cytochrome *b* sequences of the smallest mtDNA clade that included samples from the northern and southern sides of the Tokara Gap using Fu (1997)'s F_S with 10,000 coalescent simulations in Arlequin v3.5 (Excoffier & Lischer 2010). A p-value < 0.02 was interpreted as significant at the 5% level according to Fu (1997). In addition, haplotype compositions of the cytochrome *b* and RAG1 genes were compared among the island populations around the Tokara Gap.

3. Results

3.1. Sequencing

For the nuclear loci, 670, 1002, 547, 470, 655, 1408, and 478 bp of *BDNF*, *MKL1*, *PRLR*, *PTGER4*, *R35*, *RAG1*, and *SNCAIP*, respectively, were successfully sequenced for all eight *A. pellopleurus* and four *A. chinensis* samples examined for the scincid phylogeny (Appendix S2). For cytochrome *b*, we sequenced 910 bp of 161 *A. pellopleurus* and one *A. chinensis*, and detected 84 haplotypes of *A. pellopleurus* that were distinct from each other and the *A. chinensis* haplotype (Appendix S1). The haplotype composition at each sampling site is shown in Table 1. We confirmed absence of insertion/deletions and stop codons in the aligned sequences for all loci. For *A. pellopleurus RAG1*, we sequenced 1,408 bp for 72

samples. All samples were successfully phased without ambiguity [posterior probability (PP) > 0.9], and 23 haplotypes were detected (Appendix S1). Neither stop codons nor insertion/deletions were observed for *RAG1*.

3.2. Phylogenetic relationships and divergence times

In the nuclear DNA-derived outgroup phylogeny (Fig. 2), monophyly of *Ateuchosaurus* and *A. pellopleurus* were strongly supported (PP = 100%, Appendix S5). This genus was recovered as the sister clade of the *Sphenomorphus* group, which was represented by *Sphenomorphus* Fitzinger, 1843 and *Scincella* Mittleman, 1950, with strong support (PP = 100%). The 95% highest posterior density (HPD) interval of divergence time between *A. pellopleurus* and *A. chinensis* was 10.75–31.49 million years ago (MYA) (median: 19.70 MYA). The 95% HPD of divergence time between the samples from northern and southern areas was 1.94–6.54 MYA (median: 3.75 MYA).

We showed the ingroup phylogeny using the mtDNA dataset in Fig. 3 and Appendix S6. Within *A. pellopleurus*, the primary divergence was between the two major clades, A (PP = 100%) and B (PP = 100%), which corresponded to the northern and southern clades in the outgroup phylogeny, respectively. Clade A consisted of the island samples from the Amami Group (sites 10–18, Fig. 1), Tokara Group (sites 4–9), and Osumi Group (sites 1–3), and diverged into three subclades (A1–A3) with a divergence time of 0.62–1.77 MYA (median: 1.15 MYA) and marginal support (PP = 93%) for the relationship among them. Subclade A1 (PP = 100%) consisted of samples from the Osumi and Tokara Groups (sites 1–9). Subclade A2 (PP = 100%) consisted of samples from the Amami Group excluding those from Tokunoshima and Okinoerabujima Islands (sites 10–16). Subclade A3 (PP = 100%) consisted of samples from Tokunoshima (site 17) and Okinoerabujima (site 18) Islands. The samples of

northern (sites 1–7) and southern (sites 8 and 9) sides of the Tokara Gap exhibited very little genetic divergence and had a shared haplotype (HWa).

Clade B consisted of island samples from Okinawa Group (sites 19–37) and included four subclades (B1–B4 in Fig. 3). Clade B also diverged into subclades B1 (PP = 100%) and B2–B4 (PP = 97%) at a divergence time of 1.42–3.82 MYA (median: 2.53 MYA). Subclade B1 consisted of samples from Iheyajima and Izenajima Islands (sites 19 and 20). Subclades B2–B4 diverged into subclades B2 (PP = 100%) and B3–B4 (PP = 95%) at a divergence time of 1.15–3.19 MYA (median: 2.09 MYA). Subclade B2 consisted of samples from the northern part of Okinawajima Island and adjacent islets (sites 26–28 and 35–36). Subclades B3–B4 diverged into subclades B3 (PP = 100 %) and B4 (PP = 100 %) at a divergence time of 0.89–2.53 MYA (median: 1.63 MYA). Subclade B3 consisted of samples from the southern part of Okinawajima Island and an offshore islet (sites 21, 22, 29–34, and 37). Subclade B4 consisted of the island samples from Tonakijima, Agunijima, and Kumejima Islands (site 23–25).

The statistical parsimony network of the 23 *RAG1 A. pellopleurus* haplotypes is shown in Fig. 4. In the network, two distinct clusters separated by six mutational steps were recognized (RA and RB, Fig. 4). The nine haplotypes of the RA cluster were specific to the Osumi, Tokara, and Amami Groups (sites 1–18), whereas the other 14 haplotypes of the RB cluster were specific to the Okinawa Group (sites 19–36).

3.4. Haplotype composition and population genetic analysis of the Tokara samples

The smallest mtDNA clade encompassing the northern and southern sides of the Tokara Gap was the subclade A1 occurring in sites 1–9. At these sites, eight cytochrome *b* gene haplotypes and one *RAG1* haplotype were detected (Table 1). Of the eight cytochrome *b*

haplotypes, HWa was observed from most of the islands (sites 1–6, 8, and 9), whereas four (HL8a, HL8b, HL8c, and HL9) and three (HL5a, HL5b, and HL7) local haplotypes were observed from islands in the southern (sites 8 and 9) and northern parts (sites 5 and 7) of the Tokara Gap, respectively. The neutrality test of Fu's F_S was performed for the cytochrome *b* dataset of subclade A1. Fu's F_S showed a significant negative value ($F_S = -5.621$, $P < 0.00001$), which indicated recent sudden population expansion.

4. Discussion

4.1. Phylogenetic positions of Ateuchosaurus *species*

The present result of the scincid outgroup phylogeny unequivocally clarified the monophyly of the genus *Ateuchosaurus*, and the sister relationship of *Ateuchosaurus* and a lygosomine clade *Sphenomorphus* group. Although the monophyly of *Ateuchosaurus* was unsurprising given the stable taxonomic treatment with well-defined diagnoses (e.g., Mittleman 1952; Truong et al. 2008; Hedges 2014), this result provided the first molecular support for the monophyly.

However, the phylogenetic position of *Ateuchosaurus* among scincid genera has been controversial. *Ateuchosaurus* is usually placed in Lygosominae *sensu* Greer (1970, 1986) based on osteological characteristics (Greer 1970, 1986; Greer & Shea 2000). However, Greer & Shea (2000) noted that the phylogenetic position of *Ateuchosaurus* needs further consideration, because members of this genus possess a "chalcidine" head scale pattern, which is not found in any other lygosomine skinks but widely shared by many non-lygosomine genera. In preceding molecular phylogenies, the position of *Ateuchosaurus* was

317 inconsistent and had weak support, and it was sometimes excluded from the lygosomine skink
318 clade (Austin & Arnold 2006; Pyron et al. 2013; Zheng & Wiens 2016). Thus, the
319 phylogenetic position of *Ateuchosaurus* has been disputed despite their importance for
320 understanding morphological diversification of scincid lizards. The present analysis clarified,
321 with strong support (PP = 100%), that *Ateuchosaurus* is sister to the *Sphenomorphus* group in
322 the lygosomine clade. This finding indicates that the lygosomine osteological characters of
323 *Ateuchosaurus* were inherited from the common ancestor of the crown lygosomine skinks,
324 whereas the chalcidine scale pattern was convergently acquired by *Ateuchosaurus* and many
325 other skinks, as previously discussed for non-lygosomine genera (Whiting et al. 2003;
326 Brandley et al. 2005).
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328 *4.2. Historical distribution of* A. pellopleurus *and its implications for biogeography of the*
329 *Central Ryukyus*
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331 The significantly negative value of Fu (1997)'s F_S indicated recent population expansion
332 of the Tokara and Osumi Group populations. These populations showed very little geographic
333 differentiation, with widely shared haplotypes in both the mtDNA and *RAG1* datasets (Table
334 1); this demonstrated recent range expansion in this region. In addition, local genetic
335 polymorphisms in mtDNA seemed to be concentrated in the southern part of this range (Table
336 1), which can be interpreted as a footprint of recent northward founder events. Since the
337 islands in the northern and southern sides of the Tokara Gap were separated by the sea strait
338 for a long time (Machida et al. 2001), the ancestral population was limited to the southern side
339 of the gap (sites 8 and 9), and the northern populations were established by recent northward
340 dispersal, as speculated by Ota et al. (1999).
341 The present geographic distribution of this species largely ranges along both sides of the

Tokara Gap, which is superficially different from the general biogeographic pattern of terrestrial reptiles in the Ryukyu Archipelago (e.g., Ota 1998; Okamoto 2017). However, the present results indicated that the range of this species was limited to Okinawa, Amami, and southern Tokara Groups before recent dispersal. Therefore, this species should be considered endemic to the Central Ryukyu region. Although this was assumed *a priori* in preceding biogeographic discussions (e.g., Ota 1998; Okamoto 2017), these results provide the first explicit justification of this assumption.

Okamoto (2017) discussed that most Central Ryukyu endemics can be categorized as young, having been isolated in the late Miocene, and the old relicts that were isolated in the Eocene. The latter includes two genera with relictual geographic ranges, *Geoemyda* Gray, 1834 and *Goniurosaurus* Barbour, 1908, which have congeners limited to southern China to northern Vietnam. Okamoto (2017) noted that *Ateuchosaurus* shows similar geographic occurrences and may be another old relict. However, the present results indicated that *A. pellopleurus* diverged from *A. chinensis* 10.75–31.49 MYA, which largely overlaps with the divergence time of the young endemics with a Miocene origin (around 6–14 MYA; Okamoto 2017). Therefore, *A. pellopleurus* should be considered young based on divergence time congruence.

As noted by Okamoto (2017), the geographic ranges of sister taxa of younger endemics varied across taxa, such as *Ovophis okinavensis* (Boulenger, 1892), which has sister species limited to Taiwan, and *Takydromus smaragdinus* (Boulenger, 1887), which has two or more equally diverged congeners in surrounding areas, including the Japanese main islands, the Sakishima Group, Taiwan, and the continent. Then, Okamoto (2017) found that the Central Ryukyus were simultaneously isolated from surrounding areas, although he did not mention any specific geological events. The isolation of the young endemics with late Miocene origins can be explained by the formation of the Okinawa Trough, which is the back-arc basin along

the northwestern side of the Ryukyu Archipelago that formed in late Miocene to early Pliocene. Before the formation of the Okinawa Trough, the Central Ryukyus were a peripheral part of the continent. The opening of the Okinawa Trough was immediately followed by formations of the Kerama and Tokara Gaps (Miki 1995; Machida et al. 2001). Thus, the geohistory indicates that the Central Ryukyu region was simultaneously isolated from the continent, and northern (the Japanese main islands) and southern (the Sakishima Group and Taiwan) regions. Thus, the geohistory is consistent with the scenario postulated by Okamoto (2017).

The geographic range of *A. chinensis* encompasses the East China Sea coastal area of southern China. Although this region currently seems distant from the Central Ryukyus (Fig. 1A), it was directly connected to the Central Ryukyus before the Okinawa Trough opened. Therefore, the geographic ranges of *A. pellopleurus* and *A. chinensis* should be considered a result of simple allopatric speciation caused by the Miocene isolation of the Central Ryukyus, rather than relictual disjunct ranges. Thus, the geographic range of *A. chinensis*, the sister species of *A. pellopleurus*, and its divergence time may not have deviated from the general pattern of the Central Ryukyu endemics, with late Miocene origins found by Okamoto (2017).

4.3. Intraspecific variation of A. pellopleurus

4.3.1. Divergence between the Okinawa and Amami Group populations

The intraspecific variation results for mtDNA and *RAG1* collectively demonstrated distinct genetic divergence of *A. pellopleurus* between the northern lineage that occurs in the Amami, Tokara, and Osumi Groups (sites 1–18, Fig. 1), which was characterized by clade A mtDNA and *RAG1* haplotypes of the RA cluster, and the southern lineage in the Okinawa

Group (sites 26–34), which was characterized by the clade B mtDNA and *RAG1* haplotypes of the RB cluster. This geographic pattern differs from the continuous geographic morphological variation suggested by Ota et al. (1999). Although Ota et al. (1999) also recognized differentiation between the Okinawa Group and Amami–Tokara–Osumi Group populations based on geographic variation of morphological characters, they found a morphologically intermediate population at a geographically intermediate island (Okinoerabujima Island, site 18). In contrast, the present molecular results showed that the Okinoerabujima samples (N = 7 for mtDNA and N = 5 for *RAG1*) exclusively possessed alleles specific to the northern lineage (Table 1, Appendix S1), which negated the proposed intermediate status. These results thus elucidate that each of the northern and southern lineages can receive distinct species status; their diagnostic characters should be clarified by future taxonomic study.

Genetic divergence between the Amami and Okinawa Group populations was also found in most endemic reptiles and amphibians in the Central Ryukyus (Matsui et al. 2005; Tominaga et al. 2010, 2014, 2015, 2019; Honda et al. 2012, 2014; Kurita & Hikida 2014b; Kaito & Toda 2016; Shibata et al. 2016; Kaito et al. 2017). Kaito & Toda (2016) noted that these divergence times were not congruent despite the superficial congruence of biogeographic patterns, and concluded that these divergences were not caused by a common vicariance event. Tominaga et al. (2015) observed that the phylogenetic positions of the Okinoerabujima (site 18) and/or Yoronjima (the small island between site 18 and area C in Fig. 1B) populations were not concordant across taxa. The *A. pellopleurus* (this study) and *Buergeria japonica* (Tominaga et al. 2015) populations on these islands are closely related to the Amami Group populations, whereas those of *Plestiodon* Duméril & Bibron, 1839 (Kato et al. 1994; Kurita & Hikida 2014b) and *Microhyla okinavensis* Stejneger, 1901 (Tominaga et al. 2019) are closely related to the Okinawa Group populations. Thus, these islands can be

417 interpreted as a biogeographic intermediate zone between the Amami and Okinawa regions
418 that was formed by repeated dispersal by sea current or past land connections. The
419 phylogeographic studies of the species occurring in the Okinoerabujima and/or Yoronjia
420 exhibited similar shallow divergences from their closest populations in northern (*A.*
421 *pellopleurus* and *B. japonica*) or southern (*M. okinavensis* and *Plestiodon marginatus*
422 Hallowell, 1861) areas. These results suggest recent bidirectional dispersals of these taxa.
423 Repeated gene flow by such dispersal events may have occasionally caused various degrees
424 of genetic differentiations and incongruent geographic boundaries between the Amami and
425 Okinawa assemblages. The inconsistency of divergence times across taxa pointed out by
426 Kaito & Toda (2016) can be explained by repeated gene flow events between these island
427 groups, despite unclear geographic history.
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429 *4.3.2. Diversification in the Okinawa Group*
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431 Recent progress in molecular phylogeographic studies of amphibians and reptiles in the
432 Okinawa Group has revealed strong endemicity of the populations in two peripheral parts of
433 this region, Iheyajima and Izenajima (sites 19 and 20, subclade B1), and Kumejima and
434 adjacent islets (site 23–25, subclade B4). For Iheyajima and Izenajima populations, *A.*
435 *pellopleurus* showed genetic divergence from the Okinawajima populations (sites 26–34),
436 with divergence times of 1.42–3.82 MYA (Fig. 3). Divergences around 2.5 MYA between the
437 Iheyajima–Izenajima and Okinawajima populations were known for *Microhyla okinavensis*
438 (around 2.1 MYA; Tominaga et al. 2019) and *Goniurosaurus kuroiwae* (Namiye, 1912) sensu
439 lato (1.7–3.5 MYA; Kurita et al. 2018), although the latter species is absent from Izenajima.
440 However, other taxa showed older divergence times (Kaito & Toda 2016) or no significant
441 divergences between these islands' populations (e.g., Kurita & Hikida 2014b; Tominaga et al.

2015). Although *Sinomicrurus japonicus* (Günther, 1868) showed differentiation between the Iheyajima and Okinawajima populations with a comparable divergence time of 2.38–4.13 MYA, the Izenajima population of this species was genetically intermediate between Iheyajima and Okinawajima populations (Kaito et al. 2017).

In *A. pellopleurus*, divergence of the Kumejima (site 25) populations from the Okinawajima populations (sites 26–34) with divergence times of 0.89–2.53 MYA was shown (Fig. 3). Several taxa showed comparable divergence times around 1 MYA, such as *P. marginatus* (0.93–2.17 MYA; Kurita & Hikida 2014b), *Hebius pryeri* (Boulenger, 1887) (0.40–1.03 MYA; Kaito & Toda 2016), and *Sinomicrurus japonicus* (0.62–1.31 MYA; Kaito et al. 2017), whereas other taxa showed older divergence times (Honda et al. 2014) or little differentiation between Kumejima and Okinawajima populations (Tominaga et al. 2015, 2019). In addition, the phylogenetic positions of populations on the geographically intermediate island of Tonakijima (site 24) vary among taxa (Honda et al. 2014; Kurita & Hikida 2014b; Kaito & Toda 2016; Kaito et al. 2017).

Although both the Iheyajima and Kumejima assemblages diverged from the Okinawajima assemblage with generally concordant divergence times, geographic boundaries of the divergent lineages were discordant among taxa. The partial concordance with discordant boundaries was likely formed by initial allopatric isolations followed by repeated secondary dispersals. During the repeated dispersals, the local genetic elements may have been swept out in some cases but occasionally retained in other cases, as discussed by Kurita et al. (2018). Although the vicariance events that isolated the Iheyajima and Kumejima assemblages are unclear, those deserve further consideration given the concordant divergence dates among taxa. In addition, dispersibility through the sea straits for each taxon should be investigated by additional data to test the above scenario.

The mtDNA dataset of *A. pellopleurus* exhibited genetic differentiation between

northern (sites 26–28, 35, and 36; subclade B2) and southern (sites 29–34 and 37; most parts of subclade B3) parts of Okinawajima, with divergence times of 1.15–3.19 MYA. Even though they contained little information, the *RAG1* results seemed to support this divergence: the northern and southern populations did not share any haplotypes except for the widespread haplotype RHWc, whereas the haplotype RHWe was shared by two local samples in northern Okinawajima. Genetic differentiation within Okinawajima was known for two other taxa, *Plestiodon marginatus* (Kurita & Hikida 2014b) and *Goniurosaurus kuroiwae* (Kurita et al. 2018). Of these, *G. kuroiwae* exhibited distinct differentiation, with a reproductive barrier between the northern and southern populations. Given that there was no significant barrier to gene flow between the northern and southern part of Okinawajima, the genetic divergence of *A. pellopleurus* might be maintained by an intrinsic reproductive barrier as in *G. kuroiwae*; however, this needs to be confirmed by further investigation.

5. Conclusion

We discussed the historical biogeography of *A. pellopleurus* and the Central Ryukyu endemics. Congruent distributional and phylogenetic patterns, and divergence time estimates played important roles in elucidating these historical biogeographic patterns. Although the roles of common geographic distributions and phylogenetic relationships have been traditionally emphasized in historical biogeography (e.g., Crisci et al. 2003), the critical importance of divergence time estimates, theoretically argued by Donoghue & Moore (2003), was exemplified by this study and preceding studies on biogeography of the Ryukyu Archipelago (Kaito & Toda 2016; Okamoto 2017). In addition, our study clarified that *A. pellopleurus* is a typical Central Ryukyu endemic reptile, despite its exceptionally wide

geographic range. This case exemplified that “pseudo-incongruence” (*sensu* Donoghue & Moore 2003) in historical biogeography can occur in distributional patterns of each species, and it can be detected by careful inspection of population genetic characteristics of local populations. Thus, this study empirically underlined the importance of using a time-calibrated phylogeny and geographic variation in population genetic characteristics to elucidate historical biogeography.

Acknowledgments

We thank Hidetoshi Ota, Masanao Honda, Naoki Koike, Tadafumi Maenosono, Yukoh Murai, Nikolai L. Orlov, Naoya Sata, Isao Takiguchi, Mamoru Toda, and Yoshiko Yamane for providing specimens used in this study; Yusuke Fuke, Mika Saiki, and Mamoru Toda for help collecting specimens; Tatatsugu Hosoya for field assistance in the Tokara Group; and Mamoru Toda for permission to use specimens stored at the University of the Ryukyus. Our gratitude is also extended to two anonymous reviewers and Alexander Kupfer for their valuable comments and suggestions on this manuscript. Fieldwork in Takeshima, Iojima, and Kuroshima Islands of the Osumi Group was carried out with permission of Mishima Village. Fieldwork in the Tokara Group was carried out with permission of Toshima Village. The animals used in this study were appropriately treated according to the Regulation on Animal Experimentation at Kyoto University under the permission numbers H2711, H2810, H2909, H3009, and 201907.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/xxx.

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Figure legends

Fig. 1. Map showing the geographical ranges of the two *Ateuchosaurus* species and the collection sites of *A. pellopleurus* specimens examined in this study. (A) The dashed line indicates the *A. chinensis* distribution (Truong et al. 2008). (B) Sampling sites in the Northern and Central Ryukyus. The dashed line denotes the distribution of extant *A. pellopleurus* populations, and each contour line indicates the geographic range of the clade or subclade associated with those in Fig. 3. (C) Collection sites in Okinawajima Island and adjacent islets. Collection site numbers correspond to the locality numbers in Table 1 and Appendix S1.

Fig. 2. A time-calibrated phylogeny of scincid lizards based on the outgroup dataset. Horizontal bars on nodes indicate 95% HPD intervals for node heights. The asterisks indicate the nodes supported by 100% posterior probabilities. See Appendix S5 for full results.

Fig. 3. Summarized Bayesian phylogeny of *Ateuchosaurus* with estimated divergence times. Horizontal gray bars on nodes indicate 95% HPD intervals for node heights. Numbers near the internal nodes show Bayesian posterior probabilities. The names of each subclade correspond to that in Fig. 1. The shaded area indicates the consensus range of divergence time for the young Central Ryukyu endemics (6–14 MYA; Okamoto 2017). See Appendix S6 for full results.

Fig. 4. Statistical parsimony network for phased *RAG1* haplotypes of *Ateuchosaurus pellopleurus*. Closed circles indicate missing haplotypes. Haplotype names correspond to those in Table 1 and Appendix S1.

Appendix S1. List of vouchers examined in the ingroup phylogeny and their localities, haplotypes, and INSDC accession numbers. The locality numbers correspond to those in Fig. 1. KUZ: Zoological Collection of Kyoto University.

Appendix S2. List of taxa, vouchers, and INSDC accession numbers for the outgroup phylogeny.

Appendix S3. The selected partitioning schemes and molecular evolutionary models for the phylogenetic analyses.

Appendix S4. The prior distributions for divergence time calibration. The node numbers correspond to those in Appendix S5.

Appendix S5. A time-calibrated outgroup phylogeny inferred by Bayesian methods using seven concatenated nuclear DNA sequences. Horizontal bars on nodes indicate 95% HPD intervals for node heights. Numbers near internal nodes indicate posterior probabilities. Nodes 1–5 are calibrated. See Appendix S4 for the associated assumptions.

Appendix S6. A time-calibrated ingroup phylogeny inferred by Bayesian methods using mtDNA sequences. The horizontal gray bars indicate 95% HPD intervals for node heights estimated by the ingroup cytochrome *b* dataset. Numbers near internal nodes show posterior probabilities. The names of tips indicate voucher IDs (see Appendix S1) followed by locality names. The clade and subclade names correspond to those in Fig. 3.

802 **Table 1**

803 Number of specimens examined for cytochrome *b* and *RAG1*, and genetic composition of each sampling sites of *A. pellopleurus*.

Locality number	Locality	Island Group	*N* (cytochrome *b*)	Haplotype (cytochrome *b*)	*N* (RAG1)	Haplotype (RAG1)
1	Takeshima Island	Osumi Group	7	HWa	4	RHWa
2	Iojima Island	Osumi Group	12	HWa	5	RHWa
3	Kuroshima Island	Osumi Group	7	HWa	3	RHWa
4	Kuchinoshima Island	Northern Tokara Group	2	HWa	1	RHWa
5	Nakanoshima Island	Northern Tokara Group	4	HWa, HL5a, HL5b	2	RHWa
6	Suwanosejima Island	Northern Tokara Group	8	HWa	5	RHWa
7	Akusekijima Island	Northern Tokara Group	2	HL7	2	RHWa
8	Kodakarajima Island	Southern Tokara Group	7	HL8a, HL8b, HL8c	6	RHWa
9	Takarajima Island	Southern Tokara Group	3	HWa, HL9	0	n/a
10	Kikaijima Island	Amami Group	5	HL10a, HL10b, HL10c, HL10d, HL10e	5	RHWa, RHL10a, RHL10b
11	Amami City, Kagoshima Prefecture (Amamioshima Island)	Amami Group	3	HWb, HL11a, HL11b	2	RHL11a, RHL11b
12	Yamato Village, Kagoshima Prefecture (Amamioshima Island)	Amami Group	2	HWb, HL12	1	RHWa, RHL12
13	Edatekujima Island	Amami Group	2	HL13a, HL13b	0	n/a
14	Kakeromajima Island	Amami Group	4	HL14a, HL14b, HL14c, HL14d	0	n/a
15	Ukejima Island	Amami Group	1	HL15	0	n/a
16	Yorojima Island	Amami Group	1	HL16	0	n/a
17	Tokunoshima Island	Amami Group	5	HL17a, HL17b, HL17c, HL17d, HL17e	4	RHWa, RHWb
18	Okinoerabujima Island	Amami Group	7	HL18a, HL18b, HL18c, HL18d, HL18e, HL18f	5	RHWa, RHWb, RHL18a, RHL18b
19	Iheyajima Island	Okinawa Group	9	HL19a, HL19b, HL19c, HL19d, HL19e	6	RHWc, RHL19

20	Izenajima Island	Okinawa Group	1	HL20	0	n/a
21	Tokashikijima Island	Okinawa Group	10	HL21a, HL21b, HL21c, HL21d	0	n/a
22	Akajima Island	Okinawa Group	1	HL22	0	n/a
23	Agunijima Island	Okinawa Group	8	HL23a, HL23b, HL23c, HL23d	3	RHWd, RHL23
24	Tonakijima Island	Okinawa Group	2	HL24a, HL24b	0	n/a
25	Kumejima Island	Okinawa Group	12	HL25a, HL25b, HL25c, HL25d, HL25e, HL25f, HL25g	4	RHWc, RHWd, RHL25
26	Kunigami Village, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	1	HL26	1	RHWc, RHWe
27	Ogimi Village, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	10	HL27a, HL27b, HL27c, HL27d, HL27e, HL27f, HL27g, HL27h	3	RHWc
28	Nago City, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	5	HL28a, HL28b	3	RHWc, RHWe, RHL28a, RHL28b, RHL28c
29	Onna Village, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	3	HL29a, HL29b, HL29c	2	RHWc, RHL29
30	Uruma City, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	1	HL30	0	n/a
31	Yomitan Village, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	1	HL31	0	n/a
32	Nakagusuku Village, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	1	HL32	0	n/a
33	Urasoe City, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	3	HWc	0	n/a

34	Naha City, Okinawa Prefecture (Okinawajima Island)	Okinawa Group	6	HWc, HL34	4	RHWc, RHL34a, RHL34b, RHL34c
35	Sesokojima Island	Okinawa Group	1	HL35	0	n/a
36	Minnajima Island	Okinawa Group	3	HL36a, HL36b, HL36c	1	RHL36
37	Hamahigajima Island	Okinawa Group	1	HL37	0	n/a

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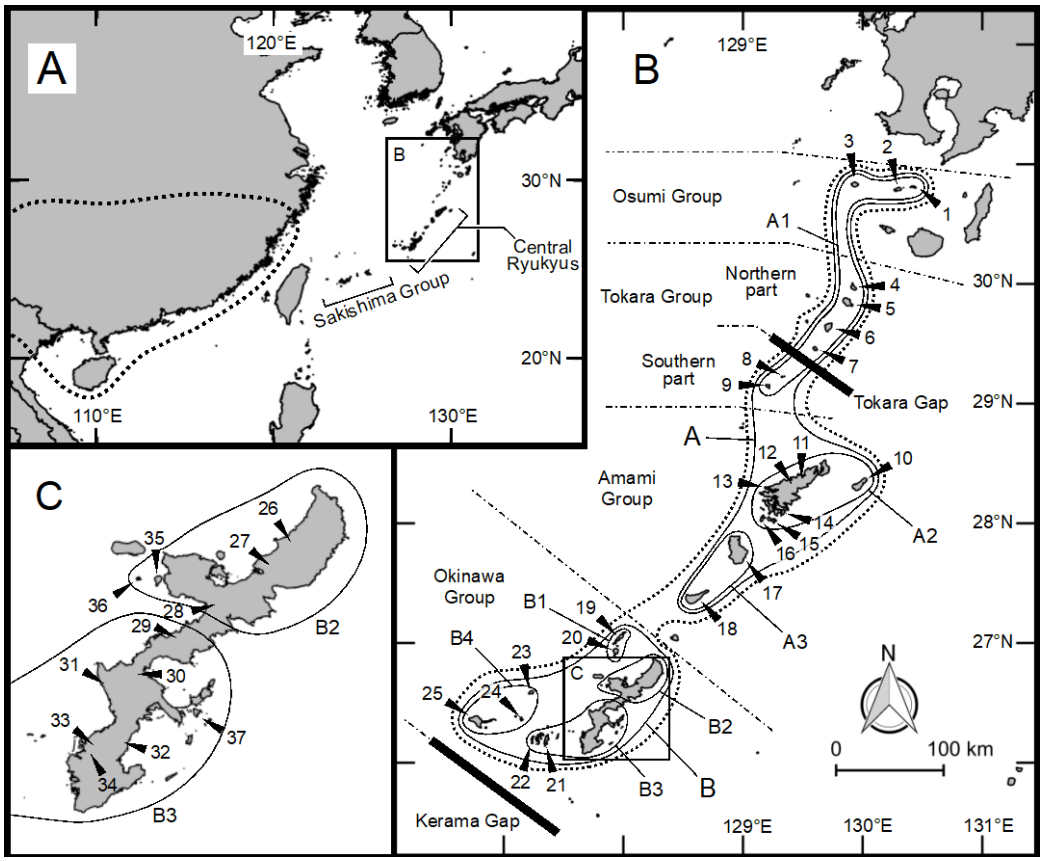


Fig. 1. Map showing the geographical ranges of the two *Ateuchosaurus* species and the collection sites of *A. pellopleurus* specimens examined in this study. (A) The dashed line indicates the *A. chinensis* distribution (Truong et al. 2008). (B) Sampling sites in the Northern and Central Ryukyus. The dashed line denotes the distribution of extant *A. pellopleurus* populations, and each contour line indicates the geographic range of the clade or subclade associated with those in Fig. 3. (C) Collection sites in Okinawajima Island and adjacent islets. Collection site numbers correspond to the locality numbers in Table 1 and Appendix S1.

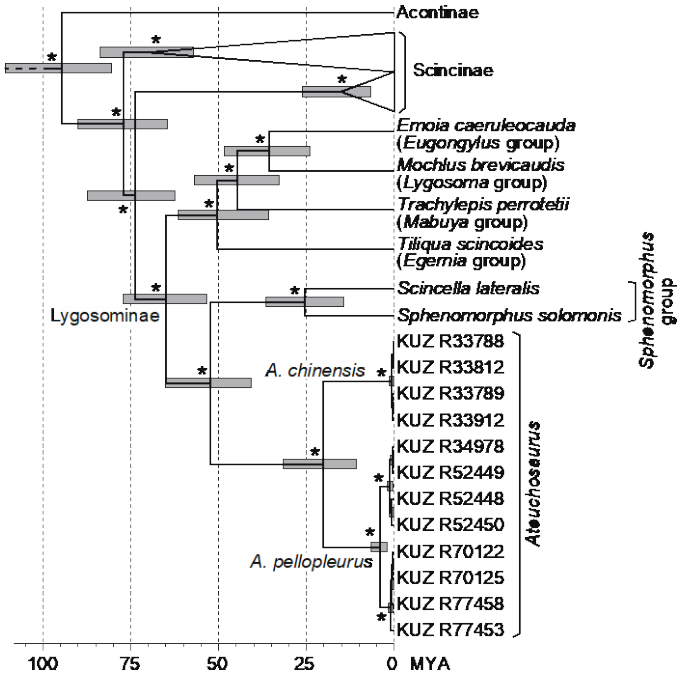


Fig. 2. A time-calibrated phylogeny of scincid lizards based on the outgroup dataset. Horizontal bars on nodes indicate 95% HPD intervals for node heights. The asterisks indicate the nodes supported by 100% posterior probabilities. See Appendix S5 for full results.

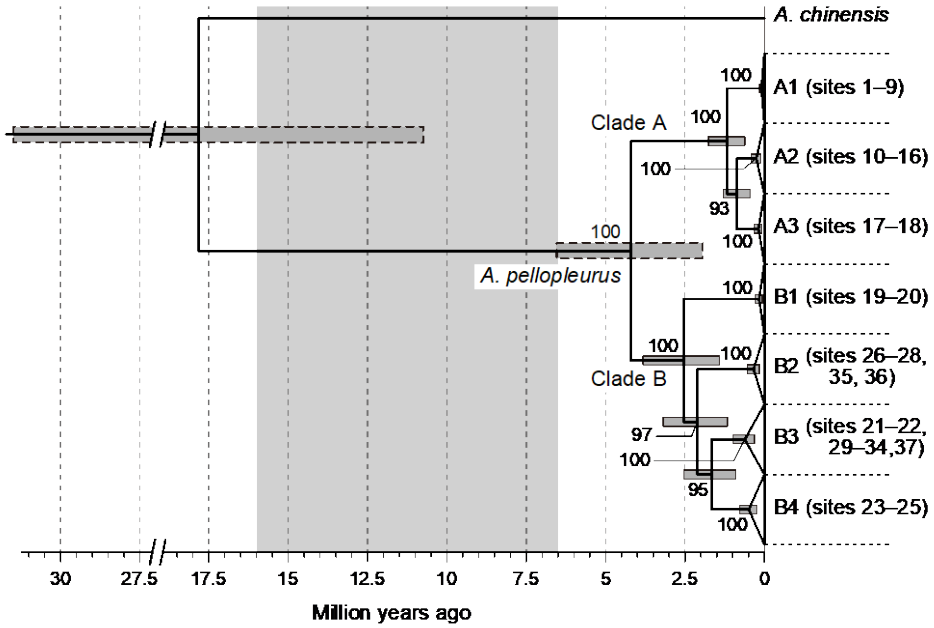
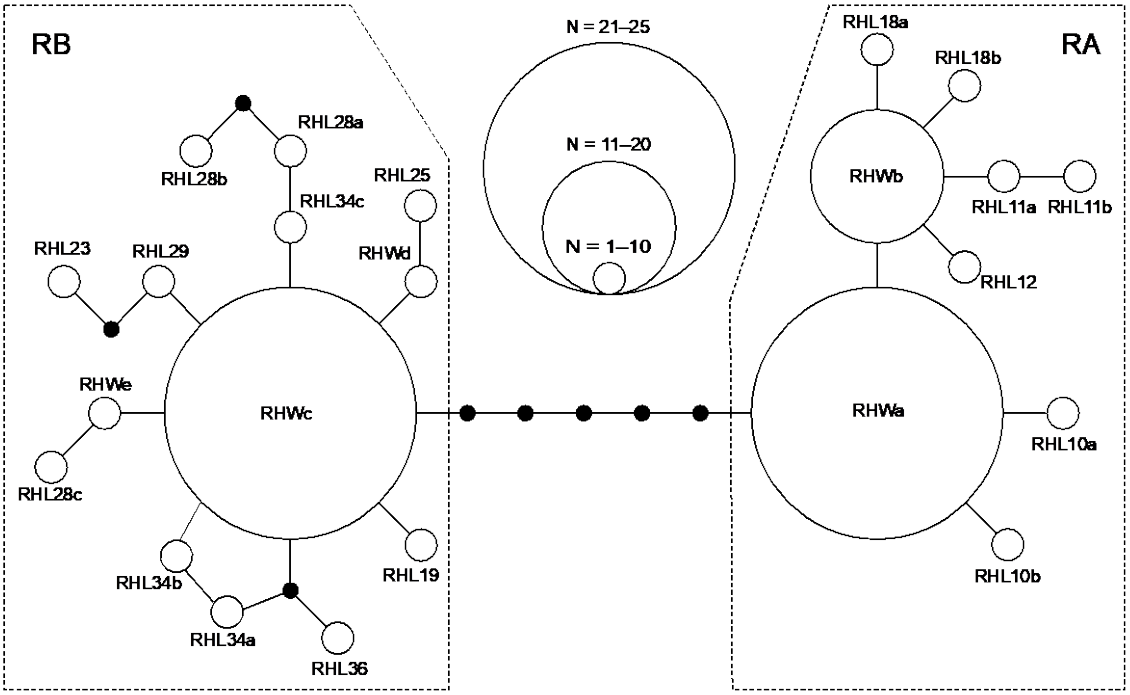


Fig. 3. Summarized Bayesian phylogeny of *Ateuchosaurus* with estimated divergence times. Horizontal gray bars on nodes indicate 95% HPD intervals for node heights. Numbers near the internal nodes show Bayesian posterior probabilities. The names of each subclade correspond to that in Fig. 1. The shaded area indicates the consensus range of divergence time for the young Central Ryukyu endemics (6–14 MYA; Okamoto 2017). See Appendix S6 for full results.



827
828 **Fig. 4.** Statistical parsimony network for phased *RAG1* haplotypes of *Ateuchosaurus*
829 *pellopleurus*. Closed circles indicate missing haplotypes. Haplotype names correspond to
830 those in Table 1 and Appendix S1.