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Lotic specialization in modern Asian newts (Caudata: Salamandridae): Phylogeny, historical biogeography, and ancestral traits based on combined DNA data

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ABSTRACT

Modern Asian newts, including *Cynops*, *Laotriton*, *Pachytriton*, and *Paramesotriton*, are primarily distributed across East Asia, displaying a broad range of environmental adaptations, including generalists inhabiting both lotic and lentic habitats and lotic specializations. Despite recent studies on their biogeographic history, how these species adapted to geographical changes remains unclear. In this study, divergence times and reconstructed ancestral traits were estimated based on phylogenetic analysis of mitochondrial and nuclear DNA sequences, totaling 4 048 bp. Results indicated that the divergence between lotic-specialized and generalist species occurred during the middle to late Miocene (95% confidence interval (CI): 16–13 million years ago (Ma)), correlating with the long-lasting arid period (14–12 Ma) and uplift of the Wuyi-Nanling mountains (15–11 Ma). Ancestral trait reconstruction revealed notable morphological changes associated with the emergence of lotic-specialized lineages. Specifically, male body size increased from 130 mm to 140 mm, and epibranchial traits shifted from thin to thick structures. Additionally, variation in tongue movability and skin texture were observed among lotic species. Each morphological shift is hypothesized to have an ecological basis, facilitating adaptation to specific environmental conditions. Overall, this study represents the first comprehensive attempt to elucidate the evolution of ancestral traits among divergent species of modern Asian newts, providing a deeper understanding of their adaptive responses to historical geographical and climatic changes.

Keywords: Modern Asian newts; Phylogeny; Divergence time; Trait reconstruction

INTRODUCTION

The family Salamandridae exhibits remarkable diversity in East and Southeast Asia, containing six genera (*Cynops*, *Echinotriton*, *Laotriton*, *Paramesotriton*, *Pachytriton*, and *Tylototriton*) and 76 species (Frost, 2023). These species represent over 50% of the global Salamandridae diversity. The six genera are distributed among distinct clades within the family. Notably, *Echinotriton* and *Tylototriton* belong to the lineage of “primitive newts” *sensu* Zhang et al. (2008), while *Cynops*, *Laotriton*, *Pachytriton*, and *Paramesotriton* belong to the lineage of “modern Asian newts” *sensu* Zhang et al. (2008). Zhang et al. (2008) and Kieren et al. (2018) reached a consensus that primitive newts and modern Asian newts originated from Europe, despite differing on estimated divergence times. Within Asia, the *Cynops*, *Pachytriton*, and *Paramesotriton* species can be separated into Terrain I and Terrain II lineages (Yuan et al., 2022), with lineage differentiation within these genera suggested to be influenced by previous weak summer monsoon episodes (Wu et al.,

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2013; Yuan et al., 2022). In addition, Luo et al. (2022) proposed that the diversification of the *Paramesotriton* lineage is closely related to Himalayan Plateau uplift and karst landscape formation in the mid-Miocene. Despite these insights, the specific adaptations of newt species to geographical and climatological changes remain poorly understood.

Compared to the primarily terrestrial primitive newts, modern Asian newts exhibit diverse aquatic adaptations, inhabiting various water bodies at different stages of their life cycle. Adult newts in the genus *Cynops* mainly inhabit and reproduce in lentic environments, although they are also frequently found to be active on land (Sparreboom, 2014) and occasionally observed in lotic environments such as streams and brooks (Inger, 1947; Marunouchi et al., 2000; Sparreboom & Ota, 1995; Sparreboom, 2014). Although field studies on *Laotriton* are limited, Stuart & Papenfuss (2002) suggested that *L. laoensis* adults inhabit stream environments with numerous pools and are likely fully aquatic based on their morphological characters, while Phimmachak et al. (2012) documented instances of infrequent overland movement. *Pachytriton* is the most aquatic genus within the family Salamandridae, with adult newts permanently residing in montane streams (Sparreboom, 2014). *Pachytriton* adults possess unique morphological features, including smooth skin, non-protruding tongues, and robust, dorsolaterally wrapping epibranchials to the neck (Nishikawa et al., 2011a), considered extreme adaptations to their aquatic habitat (Özeti & Wake, 1969). The genus *Paramesotriton*, the most diverse among modern Asian newts, primarily inhabits sluggish streams, with species such as *Par. chinensis*, *Par. fuzhongensis*, and *Par. hongkongensis* also demonstrating terrestrial activity (Fei, 2020). *Cynops* species are generally considered generalists, capable of thriving in both lentic and lotic environments, whereas species from the other three genera exhibit more specialized adaptations to lotic environments. Despite the ecological variety observed among these genera, comprehensive studies addressing the systematic, ecological, and morphological evolution of these newts remain scarce.

Advancements in species delimitation methods have revealed high species diversity within modern Asian newts, with an increasing number of species documented since 2000. However, the number of recognized genera has remained relatively stable since 1935, with only minor changes, such as the addition of subgenera and minor controversy in the synonymization of *Hypselotriton*. Furthermore, the phylogenetic relationships among these genera remain unsolved despite extensive molecular and morphological analyses. Wake & Özeti (1969) initially grouped *Cynops*, *Hypselotriton* (currently, *Cynops*), and *Pachytriton* together, while placing *Paramesotriton* in a different subgroup alongside *Triturus* and *Neurergus* based on morphological similarities. This classification was challenged by Hayashi & Matsui (1988), who applied molecular data to show that *Paramesotriton* was far closer to *Cynops* than to *Triturus*, despite the study only including three species of *Cynops* and *Paramesotriton*. Subsequent phylogenetic analyses by Titus & Larson (1995) using mitochondrial DNA cytochrome b sequences supported the monophyly of *Cynops*, *Pachytriton*, and *Paramesotriton* clades, but did not resolve the relationships within the clades. Later studies, incorporating more species and longer sequences, produced conflicting results (Figure 1) regarding whether *Cynops* should be considered as monophyletic (Steinfartz et al., 2007; Weisrock et al., 2006; Zhang et al., 2008) or paraphyletic (Arntzen et al., 2015; Chan et al., 2001; RNA-seq results of Rancilhac et al., 2021), and whether *Laotriton* should be positioned basal to *Paramesotriton* and *Pachytriton* (Arntzen et al., 2015; Weisrock et al., 2006), as a sister group to *Pachytriton* (Zhang et al., 2008), or as a sister group to *Paramesotriton* (Gu et al., 2012; Veith et al., 2018).

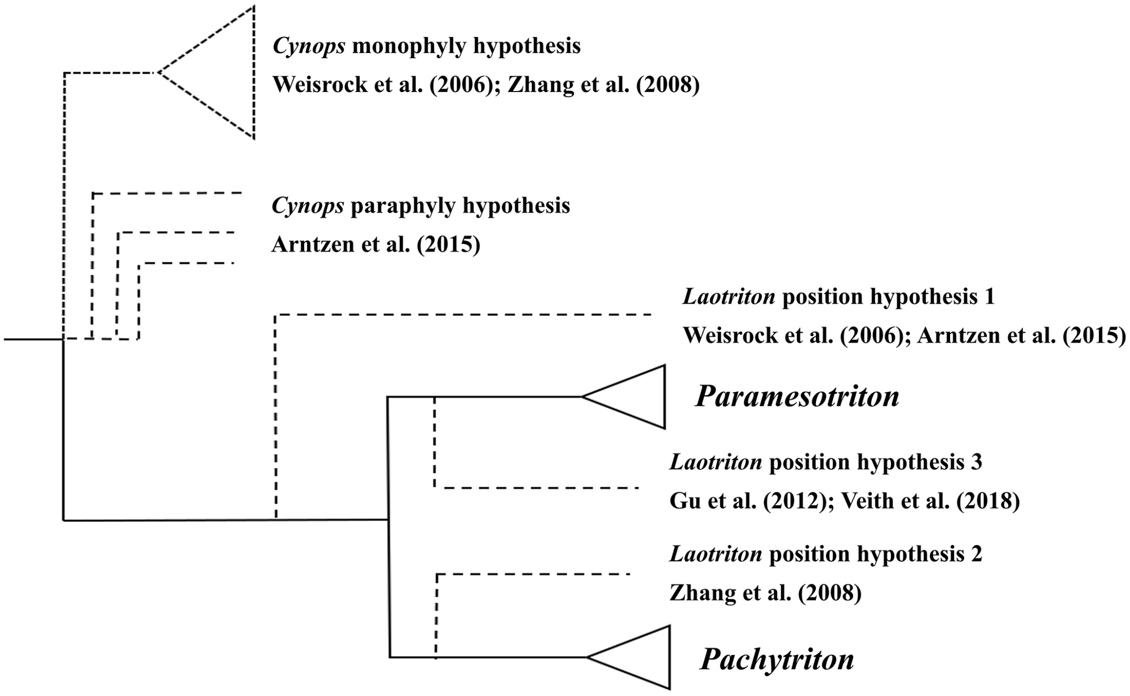

Figure 1 Overview of phylogenetic relationships in modern Asian newts based on previous studies

Solid lines refer to relatively strongly supported sister relationship between *Pachytriton* and *Paramesotriton*. Broken lines refer to conflicting hypotheses, including monophyly or paraphyly hypothesis of *Cynops* and three different position hypotheses of *Laotriton*.

In this study, a comprehensive phylogenetic analysis was performed, divergence times were estimated, and ancestral traits of modern Asian newts were reconstructed using mitochondrial and nuclear DNA sequences. This study aimed to identify potential geographic and climatic factors driving lotic specialization in these newts and to elucidate their adaptation history in response to environmental changes.

MATERIALS AND METHODS

DNA extraction and sequence amplification

Liver and muscle samples were preserved in 99% ethanol and stored at the Graduate School of Human and Environmental Studies, Kyoto University, and the Chengdu Institute of Biology, Chinese Academy of Sciences. A total of 34 specimens from 23 species were analyzed, covering all genera and approximately 64% of all modern Asian newt species (Supplementary Table S1).

Full genomic DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit (Valencia, CA, USA). Two mitochondrial fragments (16S rRNA and ND2) and two nuclear fragments (POMC and SACS) were amplified. 16S rRNA, ND2, and POMC were amplified using the touch down polymerase chain reaction (PCR) protocol, while SACS was amplified using the nested PCR protocol (Supplementary Table S2). These experiments were performed in both Japan and China.

Sequence alignment and phylogenetic analysis

All sequences were read using a 3130 Genetic Analyzer (Applied Biosystems) and aligned using MEGA-X (Kumar et al., 2018). Sequence data of five species obtained from GenBank served as the outgroup. Maximum-likelihood (ML) and Bayesian inference (BI) methods were used for phylogenetic analysis. Both model selection and ML analysis were performed using W-IQ-tree (Trifinopoulos et al., 2016), with 100 standard bootstrap replications set to test branch support. For BI analysis, model selection was carried out using Kakusan v.4 (Tanabe, 2011) and trees were constructed using MrBayes v.3.1.2 (Huelsenbeck et al., 2001), with Markov chains conducted for 10 million generations. A node was considered significantly supported when the bootstrap value was >80% (Minh et al., 2013) and posterior probability was >0.95 (Erixon et al., 2003).

Divergence time estimation

Divergence times among modern Asian newts were calculated using BEAST v.2 and BEAUti v.2 (Bouckaert et al., 2014) based on the mitochondrial regions 16S rRNA and ND2. The substitution model was set to GTR and HKY based on the model test results obtained by Kakusan v.4 (Supplementary Table S3). The clock model was configured to a strict clock with a sequence divergence rate of 0.8% per million years, based on findings from a previous study on the American newt genera *Taricha* and *Notophthalmus* (Tan & Wake, 1995). Four calibration points were acquired from two fossil records and two previous studies. Separation of the most basal species, *Salamandra salamandra*, was set to 63.2 million years ago (Ma) (Zhang et al., 2008) with a normal prior. Based on fossil records of *Procynops miocenicus*, which is closely related to *Cynops*, the separation of *Cynops* and *Paramesotriton* was set to 15 Ma (Estes, 1981) with a lognormal prior. The *Par. hongkongensis-Par. deloustali* split was set to 7.5 Ma (95% confidence interval (CI): 10.8–4.2) with a normal prior (Zhang et al., 2008). *Pachytriton archospotus* and *Pac. brevipes* divergence was set to 6.8 Ma (95% CI: 9.3–4.3) with a normal prior (Wu et al., 2013). The ML tree was used as the starting tree, and Bayesian Markov chain Monte Carlo methods were applied for 100 million generations.

Ancestral trait reconstruction

Ancestral morphological traits were reconstructed based on a consensus tree generated using BEAST. Four different traits were used in the reconstruction (Figure 2). Highly variant morphological traits among modern Asian newts, including tongue movability (Nishikawa et al., 2011a; Zhang et al., 2008), skin texture (Nishikawa et al., 2011a), and epibranchial features (Wu et al., 2012), were set as the categorical data and reconstructed using the Mesquite v.3.70 likelihood ancestral states method (Maddison & Maddison, 2021). Furthermore, average body size (total length) was quoted from atlases (Fei & Ye, 2016; Shen et al., 2014; Sparreboom, 2014; Wu et al., 1987) and theses (Li et al., 2018; Phimmachak et al., 2012; Yuan et al., 2014, 2016a, 2016b) and reconstructed using the fastAnc function in the R package phytools (Revell, 2012). Average body size for males was used as only averages were available for *C. orphicus* and female data were absent for *Pac. moi*.

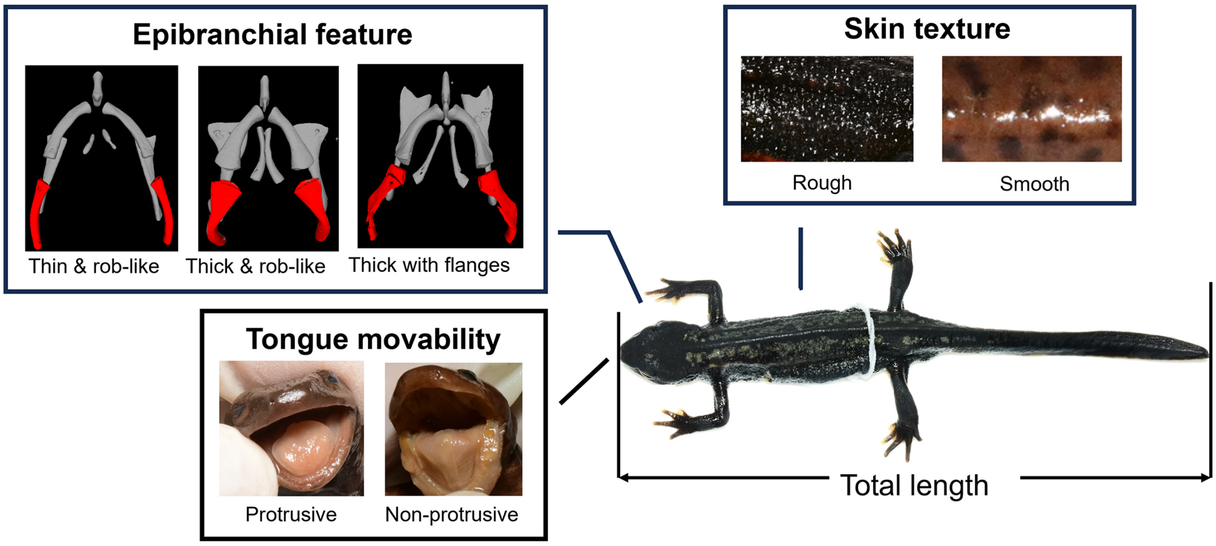

Figure 2 Morphological traits and their variations among modern Asian newts

RESULTS

Phylogenetic analysis

For most samples, sequences were obtained by combining mitochondrial 16S rRNA (maximum 1 511 bp) and ND2 (maximum 936 bp) as well as nuclear POMC (maximum 509 bp) and SACS (maximum 1 092 bp). However, some selected regions were only partially available in several samples, and one nuclear region sequence was unobtainable in six samples, possibly attributable to poor preservation conditions. Three of the five GenBank sequences lacked nuclear data and the remaining two were composite sequences from different individuals (Supplementary Table S1). The alignment of 37 samples, including outgroups, contained 4 048 columns. Among these, there were 1 636 distinct patterns and 1 396 variable sites, including 853 parsimonious informative sites and 543 singleton sites.

The ML- and BI-constructed phylogenetic trees were largely congruent across most clades (Figure 3). *Cynops* was found to be paraphyletic, occupying a basal position among modern Asian newts. Within *Cynops*, *C. pyrrhogaster* diverged first, followed by *C. ensicauda*, which separated from the continental species. The continental species, *C. orientalis* and *C. cyanurus*, formed a monophyletic group, appearing as a sister clade to other modern Asian newts, although without significant support.

The remaining three genera, *Paramesotriton*, *Pachytriton*, and *Laotriton*, were grouped together, with *Paramesotriton* showing the earliest divergence. Within *Paramesotriton*, the *Par. caudopunctatus* and *Par. chinensis* groups (Figure 3) were strongly supported as monophyletic. Within the *Par. chinensis* group, *Par. labiatus* diverged first, while *Par. deloustali*, *Par. fuzhongensis*, and *Par. yuwuensis* formed a well-supported monophyletic group, leaving *Par. hongkongensis*, *Par. chinensis*, and *Par. qixilingensis* as a non-supported group in *Paramesotriton*. Within the *Par. caudopunctatus* group, two distinct lineages with high support were identified, including the *Par. caudopunctatus* (*Par. caudopunctatus* and *Par. wulingensis*) and *Par. zhijingensis* subgroups (*Par. zhijingensis* and *Par. longliensis*).

Laotriton was positioned as sister to *Pachytriton* with significant support. *Pachytriton* contained two distinct lineages, including the *Pac. brevipes* and *Pac. inexpectatus* groups (Figure 3). Within the *Pac. brevipes* group, which contained most *Pachytriton* species, populations of *Pac. granulosus* and *Pac. brevipes* did not form a monophyletic cluster.

Divergence time

Our results suggested that divergence within the modern Asian newts likely commenced approximately 15.1 Ma (95% CI: 16.6–13.7, node 33; Table 1; Figure 4) during the middle Miocene, beginning with the initial separation of *C. pyrrhogaster* (Figure 4), followed by *C. ensicauda* approximately 14.6 Ma (95% CI: 16.1–13.1, node 32; Table 1; Figure 4). The continental *Cynops* species split from the common ancestor of the other three genera about 14.1 Ma (node 31; Table 1; Figure 4), with further divergence within these species occurring 11.8 Ma (95% CI: 13.4–10.3, node 30; Table 1; Figure 4). The first divergence among the three lotic-specialized genera (*Paramesotriton*, *Laotriton*, and *Pachytriton*) occurred approximately 12.4 Ma with the separation of *Paramesotriton* (node 26; Table 1; Figure 4). *Laotriton* and *Pachytriton* then diverged about 11.3 Ma (95% CI: 12.7–9.9, node 13; Table 1; Figure 4). Further separation of the two main *Paramesotriton* lineages, which gave rise to *Par. caudopunctatus* and *Par. chinensis* groups, occurred circa 10.6 Ma (95% CI: 11.9–9.2, node 25; Table 1; Figure 4).

Ancestral traits

Ancestral trait reconstruction suggested that the ancestors of modern Asian newts may have possessed rough skin, a protruding tongue, and thin, rod-like epibranchials (Figure 5). Average male size at the ancestral node of modern Asian newts was estimated to be 128.53 mm (Table 2; Figure 4; node 22). Lotic specialization was associated with an increase in average male size from 129.95 mm to 140.14 mm (Table 2; Figure 5; nodes 18 and 19). The shift from a protruding to a non-protruding tongue occurred in the common ancestors of

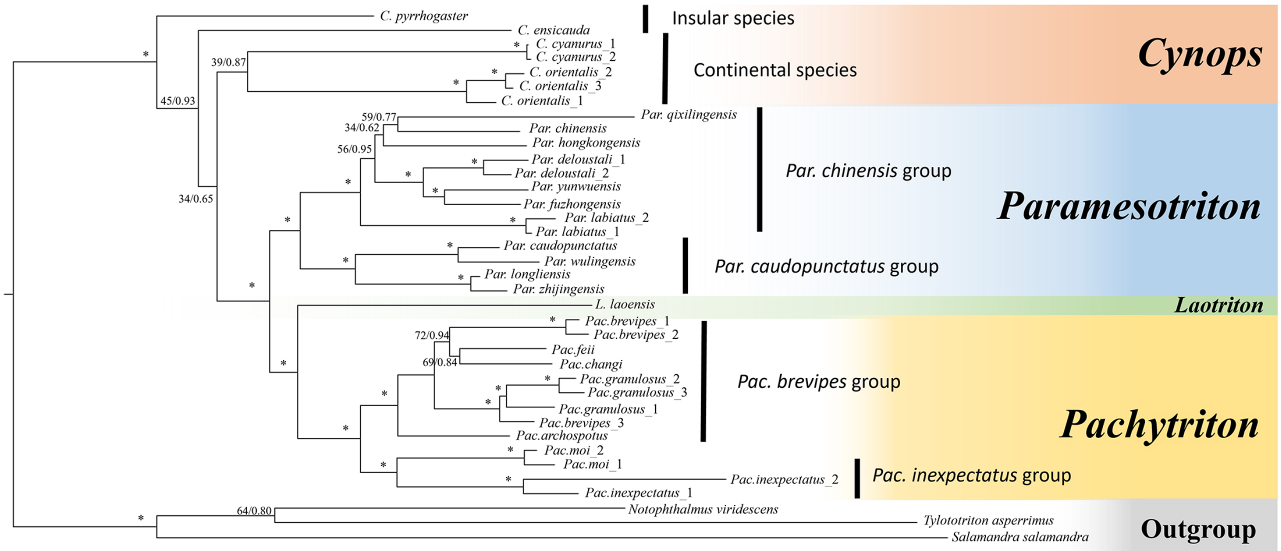

Figure 3 Phylogenetic relationships among modern Asian newts inferred from mitochondrial and nuclear sequences based on ML analysis

Numbers above branches represent ML bootstrap values and Bayesian posterior probabilities. Numbers following species name refer to localities shown in Table 1.

Table 1 Divergence time means and 95% confidence intervals calculated using BEAST

Node	Divergence time mean (Ma)	95% CI (Ma)	Node	Divergence time mean (Ma)	95% CI (Ma)
1	3.80	3.07–4.61	19	7.50	6.47–8.55
2	0.67	0.35–1.02	20	0.63	0.34–0.95
3	7.51	6.44–8.64	21	7.97	6.90–9.09
4	0.47	0.24–0.72	22	3.01	2.20–3.88
5	4.20	3.43–4.99	23	0.79	0.45–1.15
6	4.84	4.06–5.66	24	7.29	6.14–8.52
7	0.75	0.46–1.05	25	10.55	9.23–11.91
8	2.33	1.82–2.85	26	12.37	10.97–13.80
9	2.54	2.00–3.09	27	0.12	0.01–0.26
10	5.68	4.85–6.55	28	0.73	0.36–1.14
11	7.03	6.01–8.06	29	2.21	1.59–2.84
12	9.13	7.96–10.32	30	11.79	10.27–13.36
13	11.3	9.92–12.65	31	14.09	12.58–15.62
14	5.70	4.78–6.66	32	14.56	13.06–16.08
15	6.67	5.68–7.67	33	15.12	13.67–16.57
16	1.92	1.33–2.52	34	15.96	13.54–18.47
17	3.70	2.94–4.49	35	19.98	17.18–22.90
18	5.04	4.16–5.92	36	22.68	19.98–25.42

Node numbers correspond to Figure 4.

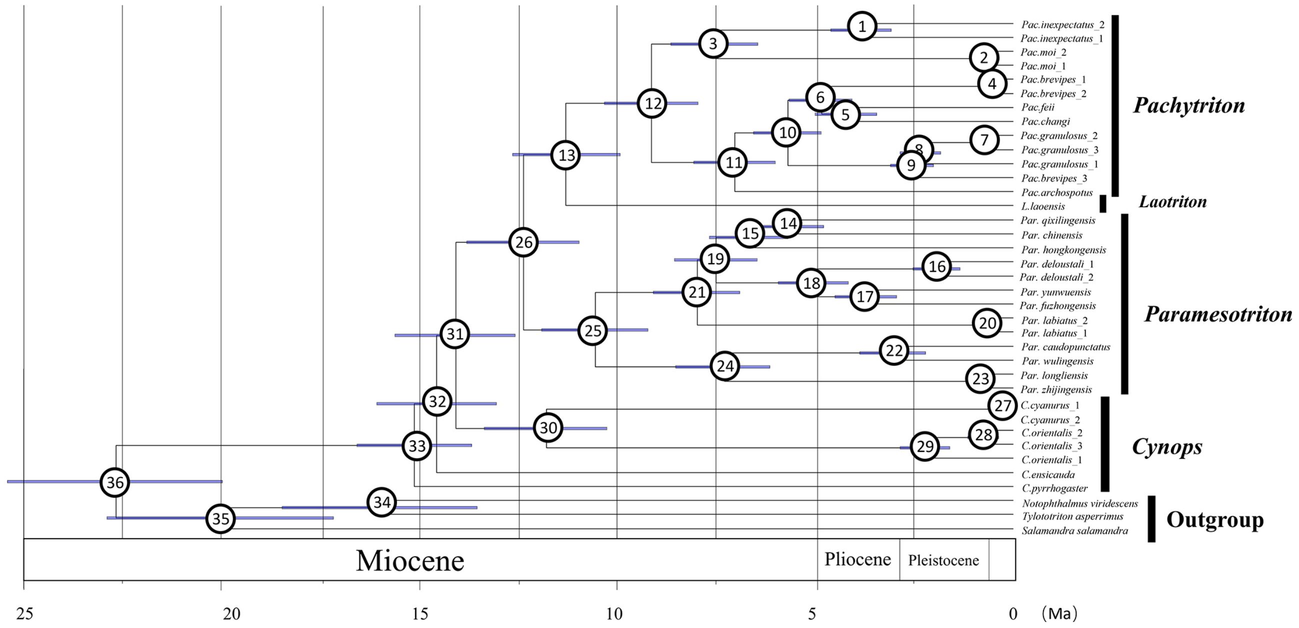

Figure 4 Estimation of divergence times for modern Asian newts

Node bars indicate divergence time with 95% CI. Number inside each pie chart refers to number of each node, as detailed in Table 1.

Pachytriton and *Laotriton*, while the shift to smooth skin was observed in *Pachytriton* species and *Par. labiatus* (Figure 5). Epibranchial features seem to have undergone several changes. The first epibranchial reinforcement occurred with the emergence of the common ancestors of *Pachytriton*, *Paramesotriton*, and *Laotriton*, with further reinforcement occurring with the emergence of *Pachytriton* (Figure 5).

DISCUSSION

Phylogenetic relationships

Phylogenetic analysis confirmed the monophyly of modern Asian newts, placing *Cynops* in the basal position relative to the other three genera. The genus *Cynops* is notably ambiguous within the Salamandridae family. Consistent with previous studies (Weisrock et al., 2006; Wu et al., 2010; Yuan et al., 2013, 2022), our findings did not establish a definitive relationship between insular and continental species. While some studies support the monophyly of continental *Cynops* species (Wu et al., 2010; Yuan et al., 2013, 2022), our results did not confirm this, possibly due to a deficiency in sampling. Historically, *Cynops* was divided into two genera: *Hypselotriton* for continental species and *Cynops* for insular species. Dubois & Raffaelli (2009) proposed treating insular and continental species as separate genera based on specific morphological differences, noting that the nasal process of the premaxilla is visible from the dorsal view of the skull in continental species, which separates the nasal bones; however, Fei & Ye (2016) observed this trait in the insular species *C. ensicauda*, challenging this classification. Furthermore, potential historical introgression has been detected between continental and insular species of *Cynops*

Table 2 Reconstruction of total length of ancestral males

Node	Ancestral male total length (mm)	Node	Ancestral male total length (mm)
1	155.23	12	142.72
2	162.59	13	153.39
3	163.30	14	143.21
4	163.42	15	142.71
5	161.78	16	141.27
6	154.65	17	138.47
7	147.40	18	140.14
8	118.56	19	129.95
9	131.91	20	129.16
10	131.80	21	129.06
11	155.16	22	128.53

Node numbers correspond to Figure 5.

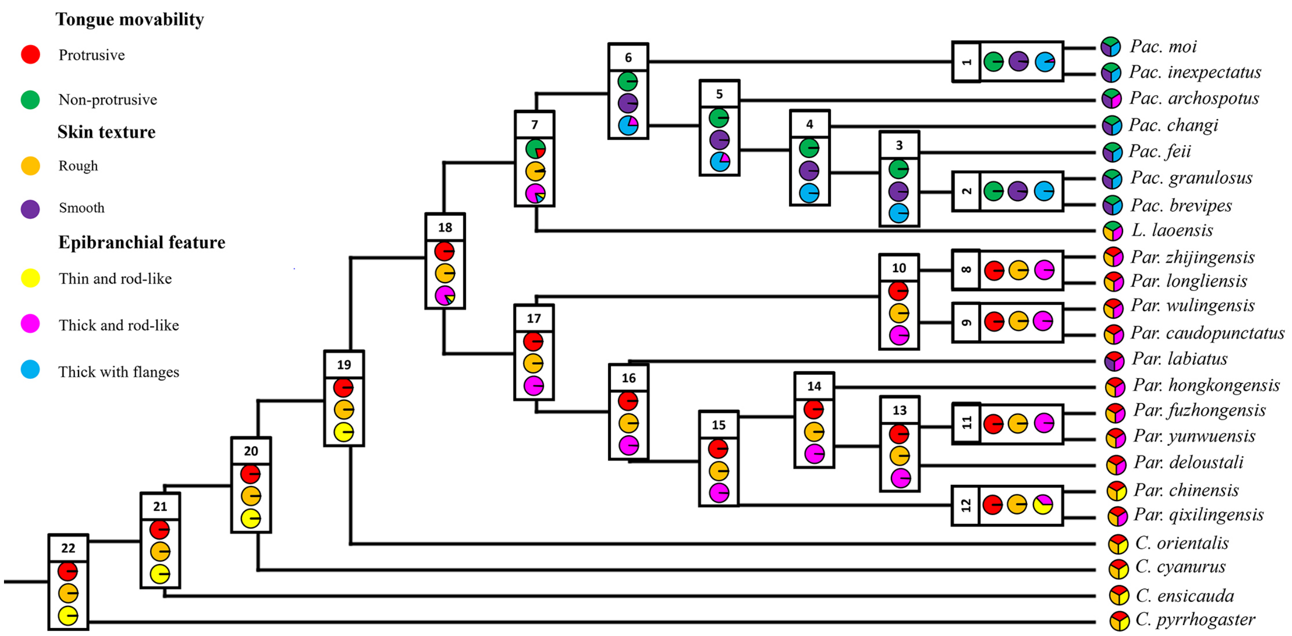

Figure 5 Ancestral trait reconstructions of tongue movability, skin texture, and epibranchial features among modern Asian newts

Outgroups are omitted from the figure. Phenotype for each species is listed in Supplementary Table 4.

as well as between continental *Cynops* and *Paramesotriton* genera (Rancilhac et al., 2021), making phylogenetic analysis based on short mitochondrial sequences unreliable. Dubois & Raffaëlli (2009) also noted differences between insular and continental species in skull shape and parotid and vertebral ridge characteristics. Further research should incorporate detailed skull landmarks and precise morphological measurements to clarify the classification and relationships between continental and insular species of *Cynops*.

Another significant phylogenetic challenge among modern Asian newts is determining the position of *Laotriton*. Initially described as *Paramesotriton* based on osteological characters (Stuart & Papenfuss, 2002), subsequent studies suggested that *Laotriton* constituted a distinct lineage separate from *Paramesotriton* (Weisrock et al., 2006; Zhang et al., 2008). Dubois & Raffaëlli (2009) further supported this distinction by assigning *Laotriton* to a new genus. Our study placed *Laotriton* as a sister group to *Pachytriton* with strong support. Stuart and Papenfuss (2002) observed that the tongue of *L. laoensis* is unique among Asian newt species, sharing similarities only with *Pachytriton* by lacking a free posterior margin, making the tongue non-protruding. This morphological trait is thought to be an adaptation for “gape and suck” feeding in aquatic environments (Özeti & Wake, 1969). Zhang et al. (2008) hypothesized that *L. laoensis* represents a transitional form between *Paramesotriton* and the more specialized *Pachytriton*. However, Nishikawa et al. (2011b) proposed that this ontogenetic change in tongue structure may have evolved in parallel in *Laotriton* and *Pachytriton*. Our results indicated a closer evolutionary relationship between *Laotriton* and *Pachytriton* than with *Paramesotriton*, suggesting that the tongue adaptations may have evolved in their common ancestor.

Biogeographic inference of habitat transition

Among modern Asian newts, the genus *Cynops* occupies the most basal position and is adapted to both lentic and lotic environments. In contrast, the remaining three genera (*Laotriton*, *Pachytriton* and *Paramesotriton*) exhibit a high degree of specialization for lotic environments. Our divergence time analyses indicated that these lotic-specialized species emerged approximately 15.6–12.6 Ma (node 31; Table 1; Figure 4), with divergence estimated at 12.4 Ma for *Paramesotriton* (node 26; Table 1; Figure 4) and 11.3 Ma for the split between *Laotriton* and *Pachytriton* (node 13; Table 1; Figure 4). During the middle Miocene, a significant global cooling event occurred (Wan et al., 2009; Zachos et al., 2008), coinciding with a shift in the monsoon system and intense aridification in the eastern Eurasia around 14–12 Ma (Dettman et al., 2003; Lu & Guo, 2014; Ma et al., 1998; Wang & Deng, 2005). This period of aridification dramatically impacted the flora and fauna of Asia (Klaus et al., 2016), leading to habitat shrinkage and the speciation of various species, such as brown frogs (Zhou et al., 2017). At the same time, the uplift of the Wuyi-Nanling mountains circa 15–11 Ma reshaped the landscape and established modern river systems in East Asia (Yan et al., 2018). This orogenic event promoted genetic diversification as populations adapted to new stable environments (Ye et al., 2016). We hypothesize that the emergence and divergence of lotic-specialized lineages among modern Asian newts may correspond to these geological and climatological changes.

Based on our estimates, speciation among the lotic-specialized genera primarily occurred in the late Miocene, approximately 9.13–5.04 Ma (nodes 3, 10–12, 14–15, 18–19, 21, and 24; Figure 4). Sun et al. (2015) identified two stepwise aridification events around 7 Ma and 5.3 Ma, driven by regional tectonic uplift. Furthermore, the modern East Asia Monsoon system developed and experienced cyclic variation during this period (An et al., 2001; Cosford et al., 2008). These climatological events have been implicated in pre-Quaternary diversification among many lineages with varying dispersal abilities in East Asia (Plants: Chen et al., 2020; Lu et al., 2020; Amphibians: Pan et al., 2017; Wang et al., 2018; Reptiles: Solovyeva et al., 2018; Aves: Jha et al., 2021; Mammals: Cheng et al., 2019; Tamma & Ramakrishnan, 2015). Our results suggest that speciation among lotic-specialized modern Asian newts may also correspond to these significant climatic and geological events.

Trait evolution accompanied by lotic specialization

Our reconstruction indicated that the evolution of traits associated with lotic specialization in modern Asian newts may have occurred periodically. In general, male body size and epibranchial traits were reinforced in the common ancestors of lotic-specialized species (Figure 5; Table 2). Ficetola et al. (2016) suggested that variations in the number of trunk vertebrae are closely related to changes in body size. Although different in detailed branches, the calibrated phylogenetic tree of Salamandridae in Arntzen et al. (2015) indicated that lotic-specialized species of modern Asian newts typically have one fewer trunk vertebra than the generalized genus *Cynops*. Changes in the number of trunk vertebrae among newts may have evolved in response to variations in precipitation, aridity, and temperature (Arntzen et al., 2015; Ficetola et al., 2016). Similar results of larger body sizes in lotic habitats have been reported in Pyrenean brook newts (*Calotriton asper*). Oromi et al. (2014) observed that stream-dwelling populations possessed a larger body size compared to those in lacustrine environments, possibly due to food scarcity and low annual temperatures in high-altitude lakes. Although both lotic and lentic habitats of modern Asian newts can be found at higher altitudes, lotic-specialized species still tend to possess larger sized bodies. This larger body size may be an adaptation to lotic factors, such as water flow resistance (freshwater drum: Rypel et al., 2006) and increased swimming ability (characid: Perazzo et al., 2019). Further research is needed to identify differences in environmental factors among lotic and lentic habitats of modern Asian newts.

In the family Salamandridae, terrestrial feeders typically exhibit cartilaginous hyoid elements and relatively complex tongue muscles, while aquatic feeders tend to possess highly ossified hyoids and reduced tongue sizes (Deban & Wake, 2000). Özeti & Wake (1969) suggested that primitive salamandrids may retain generalized feeding mechanisms for both terrestrial and aquatic environments, featuring tongues with flexible anterior parts. Our reconstruction supports this hypothesis, suggesting a generalized feeding style characterized by an ossified but weak epibranchial structure and a protruding tongue at the ancestral position of modern Asian newts (Figure 5). Further evolution of these two traits appears to have occurred independently. During the significant climatic shifts from the middle to late Miocene, moisture conditions and food availability in the terrestrial habitats of modern Asian newts likely fluctuated greatly. In contrast, aquatic habitats typically maintain more constant moisture conditions, resulting in fewer constraints on foraging and more stable prey availability for salamanders (Jaeger, 1981). Furthermore, in lotic environments, prey immigration rates may increase with current velocity (Inoue et al., 2005), but the rapid movement of prey and high water velocities may interfere with the suction feeding process. Thus, a more effective suction feeding mechanism would be advantageous in such environments. The reinforced epibranchial structure could provide additional strength and rigidity during throat expansion, resulting in a more powerful suction force (Özeti & Wake, 1969; Wu et al., 2012). Moreover, the evolution of a small, non-protruding tongue in the common ancestors of *Laotriton* and *Pachytriton* not only limited their terrestrial feeding abilities but also likely enhanced suction feeding underwater by enlarging the buccal cavity (Barrionuevo, 2016; Bramble & Wake, 1985) and reducing water flow turbulence into the mouth (Bramble & Wake, 1985; Miller & Larsen, 1989). Detailed studies on current velocity and prey availability across different habitats of modern Asian newts may provide further insights into the demands for efficient suction feeding among species.

Skin texture among salamandrids exhibits considerable variability. Most species have rough skin with tubercles, although some species undergo seasonal changes to smooth skin during their breeding season when they migrate to water (e.g., *Euproctus montanus*, *E. platycephalus*: Carranza & Amat, 2005; *Notophthalmus viridescens*: Brown et al., 1983; *Taricha granulosa*: Gibson, 1969), while other species maintain smooth skin in certain populations (e.g., *Calotriton asper*: Oromi et al., 2014). However, such seasonal or interpopulation variation in skin texture has not been reported in modern Asian newts. Instead, *Pachytriton* species and *Par. labiatus* consistently exhibit smooth skin, distinguishing them from other modern Asian newt species. This suggests that skin texture may be a stable trait within modern Asian newts, with smooth skin evolving independently in these lineages. Physiological differences between smooth and rough skin have been extensively studied in *Taricha torosa*, which undergoes seasonal changes (Harlow, 1977). Harlow (1977) found that smooth-skinned individuals in the aquatic phase had significantly lower water transport and higher sodium transport, suggesting an osmoregulatory function for these morphological changes. This function could be advantageous for an aquatic lifestyle in both lotic and lentic environments. However, among modern Asian newts, smooth skin is only observed in lotic-specialized species, suggesting a potential link between smooth skin and adaptation to lotic environments in adulthood.

CONCLUSIONS

This study investigated the phylogeny, biogeography, and trait evolution among modern Asian newts using mitochondrial and nuclear DNA from samples covering over 60% of recognized species. Both ML and BI approaches grouped the lotic-specialized species together and identified *Laotriton* and *Pachytriton* as sister genera. The evolutionary scenario of lotic specialization was revealed through divergence time estimates and ancestral trait reconstructions. Divergence time analysis indicated that the prolonged aridity during the middle to late Miocene may have triggered lotic specialization, proposing a new hypothesis for species diversification in modern Asian newts. Trait reconstructions showed that male body size increased and epibranchial structures were strengthened with the emergence of lotic-specialized lineages, suggesting that these traits may be crucial for adaptation to lotic habitats. Additionally, the evolution of extra flanges on the epibranchial structure and changes in tongue movability suggest that suction feeding efficiency may differ among lotic habitats. Furthermore, variations in skin texture may be indicative of different levels of aquatic adaptation among modern Asian newts.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Sample collections in the field were conducted following the Wildlife Protection Law of Japan, the People's Republic of China, and the Socialist Republic of Vietnam. All laboratory experiments followed the Animal Experiment Guidelines of Kyoto University and Chengdu Institute of Biology. This study was conducted under the MoU and agreements between Kyoto University and the Chinese Academy of Sciences, and LoA and agreements between Kyoto University and the National University of Laos.

DATA AVAILABILITY

Mitochondrial and nuclear sequence data have been deposited to the DNA Data Bank of Japan under Accession number LC746174–LC746205, LC746324–LC746355, LC746900–LC746957. The data set is deposited in the Science Data Bank (https://www.scidb.cn/) under DOI: https://doi.org/10.57760/sciencedb.zrdc.00004 and Genbase (https://ngdc.cncb.ac.cn/genbase/) under Accession number C_AA082893–C_AA083014.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Y.P.S. and K.N. conceived and designed the research and wrote the manuscript; K.N., J.P.J., M.M., D.Q.R., A.T., and D.S. conducted field surveys and collected samples; Y.P.S. and N.Y. performed molecular analyses; M.M., N.Y., A.T., and D.S. provided valuable suggestions and revisions. All authors read and approved the final version of the manuscript.

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