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Relationships of the Salamandrid Genera *Paramesotriton, Pachytriton,* and *Cynops* Based on Mitochondrial DNA Sequences

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We compared 786 base pairs of cytochrome *b* mitochondrial DNA sequence to examine the evolutionary relationships among seven species belonging to three genera of Asian newts: *Paramesotriton*, *Pachytriton*, and *Cynops*. We find strong evidence supporting recognition of a clade for these genera. Although bootstrap support values are relatively low, both parsimony and likelihood analyses suggest that the species of *Paramesotriton* sampled form a monophyletic group with *Paramesotriton caudopunctatus* basal to the other three species. *Cynops* appears to be paraphyletic, with *Pachytriton* and *Paramesotriton* being more closely related to *Cynops pyrrhogaster* than to *Cynops cyanurus*. *Pachytriton* and *Paramesotriton* exhibit some morphological similarities and have more specialized breeding habits and environmental requirements than *Cynops*, suggesting that they shared an evolutionary history before diverging. Our morphological investigations corroborate previous studies that suggested *Cynops* is the most generalized representative of the clade and that it retains several ancestral character states.

ASIAN newts of the genera *Paramesotriton, Pachytriton,* and *Cynops* comprise 15 currently recognized species and several undescribed species that are widely distributed in southeastern Asia, including Japan, China, and northern Vietnam (Zhao, 1999; Thorn and Raffaëlli, 2001). Phylogenetic studies of morphology (Wake and Özeti, 1969) and molecular characters (Titus and Larson, 1995) for the family Salamandridae have linked these three genera in a trichotomy with little or no resolution. Initial surveys focusing on variation in behavior, reproductive pattern, external morphology, and skull/hyobranchial characters found that the relationships among the three genera differed depending on the characters used for analyses (Wake and Özeti, 1969). These authors concluded that *Pachytriton* is most closely related to *Cynops wolterstorffi* (at the time placed in the monotypic genus *Hypselotriton*) based on overall similarity in morphology and aquatic feeding mechanisms and argued that these two might have arisen from an ancestral stock close to that which gave rise to *Cynops* (only *Cynops pyrrhogaster* was available to them). However, they were uncertain concerning the phylogenetic relationships of *Paramesotriton,* which had been considered a close relative of *Cynops* and *Pachytriton* (Freytag and Petzold, 1961; Freytag, 1962).

A more recent investigation combining molecular (mitochondrial DNA) and morphological characters was also unable to resolve these relationships (Titus and Larson, 1995). However, this study focused on higher level relationships among salamandrids and included only one species of each of our three focal genera in the molecular analyses, thereby offering limited resolution on relationships among species. Nonetheless, in the combined molecular and morphological analyses *Cynops, Paramesotriton,* and *Pachytriton* form a well-supported polytomy establishing the monophyly of this group.

Studies including multiple species from each genus have also had difficulty resolving relationships within this clade of Asian newts. An allozyme study of salamandrids including two species of *Cynops* (*pyrrhogaster* and *ensicauda*) and one species of *Paramesotriton* (*hongkongensis*) but no species of *Pachytriton* suggested that *Cynops* may be paraphyletic with respect to *Paramesotriton* (Hayashi and Matsui, 1989). Finally, a phenetic investigation of the relationships within *Paramesotriton* found evident differences in hyoid apparatus and skull characters among five species in this genus (Pang et al., 1992). Because no outgroups from other genera were used for comparison, it is difficult to determine how these results extend to further relationships among all Asian newts. The general lack of resolution in these systematic studies stems from the conservative nature of morphology in this lineage, with numerous plesiomorphic characters retained in species having an overall ''generalized'' form (Özeti and Wake, 1969).

Here we compare mitochondrial DNA (mtDNA) gene sequences from representatives of these three genera to examine the phylogenetic relationships among them. Specifically, we use molecular data to address three questions about relationships within this group using molecular data. First, we examine higher level relationships among the genera *Paramesotriton,*

Pachytriton, and *Cynops.* Second, we focus within each genus to look at relationships among species. And third, we address the possibility of the paraphyly of *Cynops.* In addition, we compare morphological data for 14 of the 15 described species and one undescribed species to identify diagnostic characters that may further clarify relationships within this clade.

Materials and Methods

Laboratory protocols.—Included in our study were 16 individuals representing four of the six species of *Paramesotriton,* one of the two species of *Pachytriton,* and two of the seven species of *Cynops.* Two species of *Triturus (vulgaris* and *carnifex)* from Europe, *Taricha granulosa* from the United States, and two species of *Tylototriton* (*taliangensis* and *verrucosus*) from southeast Asia were selected as sequential outgroups to our clade (Titus and Larson, 1995; Appendix 1). Partial cytochrome *b* mtDNA sequences for *Triturus vulgaris* and *Triturus carnifex* were obtained from GenBank (Caccone et al., 1997; accession numbers U55498 and U55499). For all other individuals, genomic DNA was isolated from frozen tissues or from samples preserved in EtOH by standard proteinase K digestion followed by either salt or phenol-chloroform purification. We used the polymerase chain reaction (PCR) to amplify approximately 690 base pairs of the cytochrome *b* region of the mtDNA with the primers MVZ16 (5′-AAA TAG GAA RTA TCA YTC TGG TTT RAT-3′) and either MVZ15 (5′-GAA CTA ATG GCC CAC ACW WTA CGN AA-3′) or Triton-cytb-Fl (5′-CAA CGC CAT CAA ACA TCT CA-3′). PCR amplification reactions were performed in total volumes of 25 μl with containing 100 ng of DNA template, 1X *Taq* buffer, 1.0 μM of each primer, 0.75 mM dNTPs, 1.5 mM $MgCl_2$, and 0.625 units *Taq* polymerase. Amplification consisted of initial denaturation at 94 C for 5 min followed by 35 cycles of denaturation for 1 min at 94 C, annealing for 1 min at 45–47 C, and extension for 1.25 min at 72 C. PCR amplifications were terminated with a final extension period of 5 min at 72 C. We used ABI fluorescent dye terminator chemistry to cycle sequence fragments in both directions with the same primers used in amplification. Products were electrophoresed on a 4.75% acrylammide gel on an ABI 377 automated sequencer (Applied Biosystems, Costa Mesa, CA).

Phylogenetic analyses.—MtDNA sequences were aligned to each other and to the cytochrome *b* sequence of *Xenopus laevis* in the program Sequencher version 3.1. Alignment was done by eye and was straightforward because no insertions or deletions were present. Amino acid translations of our sequences were compared with that of *Xenopus* (Roe et al., 1985) to ensure that there were no nonsense mutations or frameshifts. We sequenced 19 individuals of which 15 were unique haplotypes used for phylogenetic analysis (submitted to GenBank under accession numbers AF295671–AF295685). In addition, we included in our analyses the partial cytochrome *b* sequences for two species of *Triturus* (*vulgaris* and *carnifex*) obtained from GenBank.

All phylogenetic analyses of the cytochrome *b* sequences were conducted using the program PAUP*4.0beta2 (D. L. Swofford, Sinaner Assoc., Inc., Sunderland, MA, 1999, unpubl.). We assigned the two species of *Tylototriton* as outgroups for all analyses. Pairwise sequence divergences and Kimura two-parameter (K2p) corrected divergences were estimated among all pairs of sequences. We assessed levels of saturation for base substitutions by plotting percent sequence divergences against K2p distances for transitions and transversions at each codon position. Kimura two-parameter values higher than corresponding uncorrected percent sequence divergence suggest that transitions and transversions at the third codon position may be saturated (Fig. 1). Therefore, to determine the effect that saturation may have on topology, we analyzed our data using both equal weighting and with third position changes downweighted to both 25% and 50% of first and second position changes. Other than the weighting option, all other assumptions and parameters were identical in phylogenetic reconstruction.

Maximum parsimony (MP) analyses consisted of branch-and-bound searches using initial upper bound computed via stepwise addition, ''furthest'' addition sequence, and ''MulTrees'' options in effect. We also performed MP bootstrap analysis, with 1000 replicates, as a measure of internal support; the settings for bootstrap analyses were the same as those for the original branch-and-bound search.

Maximum likelihood (ML) analyses included heuristic searches with 100 replicates of random addition of sequences and one tree held at each step. For ML analyses, we selected TBR branch swapping, the ''MulTrees'' option in effect, and ''steepest descent'' option not in effect. We chose the HKY model (Hasegawa et al., 1985), with starting branch lengths obtained using Rogers-Swofford approximation and no enforcement of a molecular clock. ML bootstrap used the same settings, with the exception that

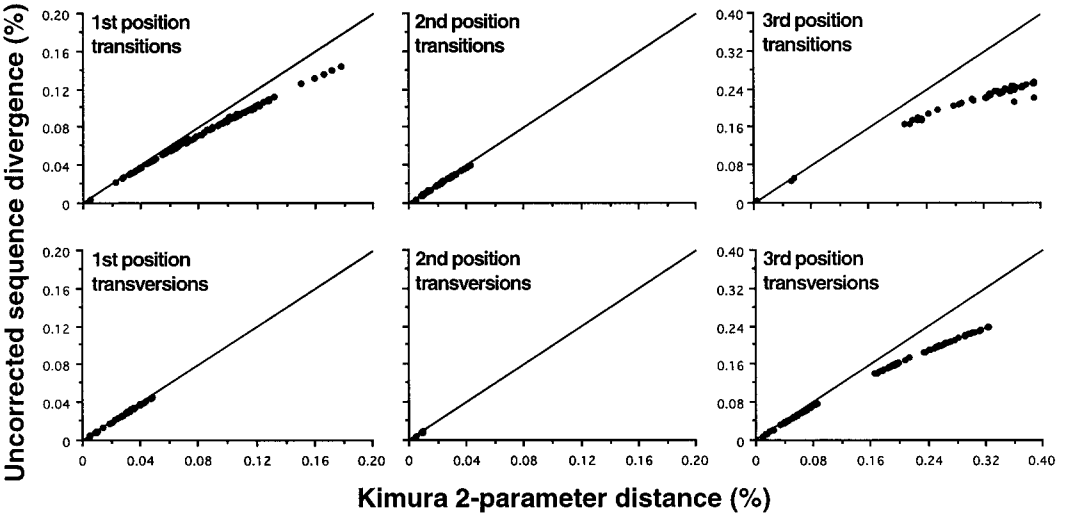

Fig. 1. Pairwise divergence plots for all individuals used in molecular analyses. Kimura two-parameter distances (x-axis) are plotted against uncorrected percent sequence divergences (y-axis) for transitions and transversions at each codon position.

we used only 10 random sequence addition replicates to decrease total analysis time.

We estimated decay indices for all branches on our parsimony tree using the program AutoDecay 4.0.2 (T. Eriksson, Stockholm Univ., 1999, unpubl.). We tested our preferred topology against alternate hypothetical trees using tree comparison tests. Tests were conducted with the most parsimonious tree under parsimony criteria using the Wilcoxon signed-ranks test (Templeton, 1983) and with the tree obtained from likelihood searches under likelihood criteria with the KH test (Kishino and Hasegawa, 1989).

Diagnostic morphological characters.—We gathered morphological data for all ingroup taxa included in our molecular analysis and most of the remaining species of *Paramesotriton, Pachytriton,* and *Cynops.* These included all described species of *Paramesotriton* (*caudopunctatus, deloustali, fuzhongensis, hongkongensis, guangxiensis,* and *chinensis*), both described (*labiatus, brevipes)* and one undescribed species of *Pachytriton,* and six of the seven described species of *Cynops* (*orientalis, orphicus, cyanurus, pyrrhogaster, wolterstorffi,* and *ensicauda*). Outgroups were excluded from morphological analyses because previous studies (e.g., Titus and Larson, 1995) and our own molecular analyses confirm the monophyly of the ingroup taxa. Data were scored from cleared-and-stained specimens and X-rays of alcohol-preserved specimens. We chose morphological characters for examination based on previous studies that had identified them as useful in discerning among salamander genera and species (e.g., Chang and Boring, 1939; Wake and Özeti, 1969; Zhao and Hu, 1988). Our goal was to examine whether these characters were diagnostic among species. Thus, we examined intraspecific variation and compared it to variation previously reported among species. We made cranial measurements, including total skull length, face skull length, neural skull length, skull breadth, and length of the maxillaries. We noted cranial characteristics, including the state of the fronto-squamosal arch, position of the maxilla relative to the pterygoid, length of the frontal processes and the degree of contact of the nasals. In addition, we determined the tarsal and carpal patterns of each limb and the number of trunk, caudo-sacral, and caudal vertebrae. Given the limited number of morphological characters, we did not code character states and subject them to phylogenetic analyses. Our objective was only to identify diagnostic characters and to possibly emphasize those that would be useful in further phylogenetic studies.

Results

We collected 19 cytochrome *b* sequences representing seven species from the three genera and three successively more distant outgroup species. We also included in our analyses previously published GenBank sequences from two *Triturus* species as additional outgroups (Cac-

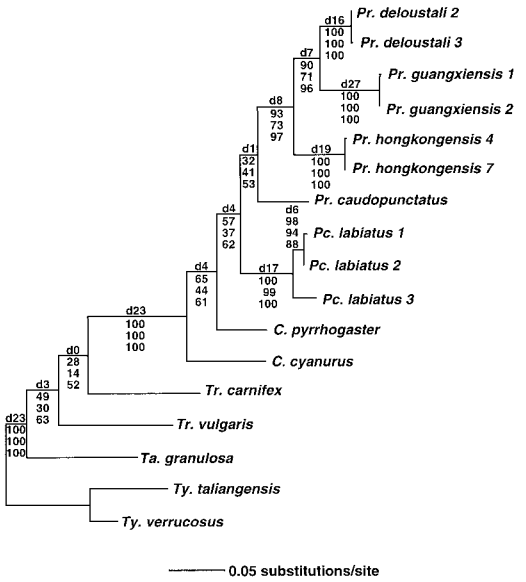

Fig. 2. Maximum likelihood phylogram for the taxa included in this study. Numbers on branches are various measures of nodal support. From top to bottom: decay indices, unweighted MP bootstraps, weighted MP bootstraps (50%), and unweighted ML bootstraps. Generic abbreviations are as follows: Pr. = *Paramesotriton,* Pc. = *Pachytriton,* C. = *Cynops,* Tr. = *Triturus,* Ta. = *Taricha,* Ty. = *Tylototriton.*

cone et al., 1997). Two *Cynops cyanurus* had identical sequences as did four of the five *Paramesotriton deloustali*; therefore, we used 17 unique sequences in our phylogenetic analyses. Of the 786 base pairs, 488 were constant, 64 were variable, and 234 were parsimony informative. Among species within ingroup genera, the largest sequence divergences were 15.8% for *Paramesotriton* and 13.2% for *Cynops.* For the three *Pachytriton labiatus,* the highest sequence divergence (K2p) was 3.5%. Sequence divergences among members of the three ingroup genera varied from 10.9% between *Pachytriton labiatus* and *Paramesotriton caudopunctatus,* to 18.9% between *Cynops cyanurus* and *Paramesotriton guangxiensis* (Appendix 2).

Maximum parsimony analysis yielded a single most parsimonious topology (L = 700; CI = 0.599; HI = 0.401). Maximum likelihood yields one most likely tree with a score of -ln L = 4248.81. MP and ML trees were identical in topology with respect to all ingroup taxa and three of the four outgroup taxa (*Taricha granulosa* and two species of *Tylototriton*; Fig. 2). They differed only in relationships among the two *Triturus* species; in MP the *Triturus* form a monophyletic group and in ML they are sequential branches at the base of the ingroup clade. However, the support values for the branch resulting in a paraphyletic *Triturus* are consistently low (bootstraps range from 14–52%). Thus, our topology does not necessarily support the paraphyly of *Triturus.* In all phylogenetic analyses, the monophyly of the *Cynops-Pachytriton-Paramesotriton* clade is well supported (for measures of nodal support, see Fig. 2). Within the ingroup clade, measures of nodal support for unweighted analyses were comparable to those with third position changes downweighted and some nodes are well supported by bootstrap and decay indices.

We find strong support for the monophyly of each of the species for which two or more populations were sampled. We record a bootstrap value of 100 for the monophyly of the following species (Fig. 2): *Paramesotriton deloustali* (16 decay), *Paramesotriton guangxiensis* (27 decay), *Paramesotriton hongkongensis* (19 decay), and *Pachytriton labiatus* (17 decay). We also see significant phylogenetic structure within *Paramesotriton* with unweighted MP bootstrap values of 90 or greater for the clade including *P. deloustali* and *P. guangxiensis* (90 bootstrap, 7 decay), and another clade including *P. deloustali, P. guangxiensis,* and *P. hongkongensis* (93 bootstrap, 8 decay). Little support is found for adding *Paramesotriton caudopunctatus* to the clade (32 bootstrap, 1 decay).

The two species of *Cynops* do not form a clade. Instead our analyses recover a clade including *Cynops pyrrhogaster* and all *Paramesotriton* and *Pachytriton* (65 bootstrap, 4 decay), suggesting that *Cynops* is paraphyletic with respect to *Paramesotriton* and *Pachytriton.*

Tree comparison tests in MP (Templeton tests) cannot reject the hypothesis that *Cynops* is monophyletic. Constraining the two species of *Cynops* to be monophyletic results in a tree only seven steps longer (L = 707, Wilcoxon signed ranks comparison to the most parsimonious tree $P = 0.17$). Maximum likelihood (Kishino-Hasegawa) tests are also unable to reject this hypothesis. Tree comparison tests result in a tree only slightly longer (-ln L = 4256.64, Kishino-Hasegawa tree comparison with the most likely tree $P = 0.17$). Nonetheless, phylogenetic reconstructions under both optimality criteria yield a topology with the basal paraphyly of *Cynops* and with moderate values of support for ML bootstrap and higher levels for MP bootstrap.

Morphological data were collected from 14 described and one undescribed species of *Paramesotriton, Pachytriton,* and *Cynops.* Members of these genera are similar morphologically, sharing the same carpal and tarsal patterns (inter-

medium and ulnare fused in the manus and distal tarsals 4 and 5 fused in the pes) as well as other cranial and skeletal characters, such as the boxlike nature of the skull with its flattened dorsal surface and grooved surface of the parietals behind the fronto-squamosal arches (Fig. 3). However, species in these three genera are not identical and variation in cranial and external morphology, especially coloration, is useful in distinguishing among them.

Of the species examined, those in the genus *Pachytriton* are the most distinct in terms of morphology. All species of *Pachytriton* have smooth skin, a relatively slender body lacking a vertebral ridge and a tail that is compressed laterally to varying degrees (see plate 6B Zhao and Hu, 1988; plates 4B and 4C Zhao and Adler, 1993). The skull of *Pachytriton* is long and narrow; the average skull breadth to skull length ratio for all adults examined is 0.66 ± 0.06. Where the relatively long maxillary bones approach the pterygoids, the elements form approximately straight lines. The fronto-squamosal arch is rarely complete and attenuate if formed. In all specimens of *Pachytriton,* the frontal process of the premaxilla, which is both long and broad, separates the nasals. The hyobranchial apparatus of *Pachytriton* is unique with the stout, bony epibranchials flaring dorsolaterally and wrapping around the neck (Özeti and Wake, 1969). One specimen of *Pachytriton* had 13 trunk vertebrae, whereas all others had 12. Caudo-sacral vertebral counts were more variable; individuals had either two or three caudo-sacral vertebrae with no species-specific pattern emerging.

All *Paramesotriton* have rough skin and a prominent vertebral ridge, often with a lateral ridge along each side of their back. The parietal ridges of the skull are prominent as well and the tail is high and laterally compressed with bony apophyses extending dorsally and ventrally from the caudal vertebrae (Fig. 3). The tips of the maxillary bones do not contact the pterygoid as in *Pachytriton*; they instead lie outside and anterior to the pterygoid, thus forming an angle rather than a straight line. The fronto-squamosal arch is complete in all specimens examined and relatively stout in all species except *P. caudopunctatus.* The nasals are well separated, and there is a long frontal process of the premaxilla. As in *Pachytriton,* most *Paramesotriton* have 12 trunk vertebrae (two individuals had 11), and the number of caudo-sacral vertebrae varies from two to three.

Several morphological characters distinguish *P. caudopunctatus* from the other species of *Paramesotriton* in this study (*P. guangxiensis, hongkongensis, deloustali,* and *chinensis). Paramesotriton caudopunctatus* is less robust, and its skull is longer and narrower (ratio of skull width to skull length of *P. caudopunctatus* = 0.70 ± 0.01) compared to the broader skulls of the other four species of *Paramesotriton* (width to length of *Paramesotriton* excluding *P. caudopunctatus* = 0.85 ± 0.11). The moderately stout and bony epibranchials of *P. caudopunctatus* are flared dorsolaterally, similar to the epibranchials of *Pachytriton,* whereas the other species of *Paramesotriton* have a hyobranchial apparatus like the one described for *P. hongkongensis* with relatively slender and nearly straight epibranchials (Özeti and Wake, 1969).

We examined six of the seven described species of *Cynops,* and whereas we found osteological variation, there is overall morphological similarity among species. All *Cynops* are smaller-bodied than either *Pachytriton* or *Paramesotriton* (Fig. 3). They have a vertebral ridge, although it is not always prominent, and almost all individuals lack lateral ridges. The tail is laterally compressed, and except for *C. wolterstorffi,* the skin is granular. Some individuals have parietal ridges, although they are generally not as prominent as those of *Paramesotriton.* In all *Cynops,* the relationship of the maxillary bone to the pterygoid is similar to that of *Paramesotriton,* with the tip of the maxilla outside and anterior to the pterygoid and with no contact between these elements. The fronto-squamosal arch was complete in all individuals, but it is somewhat attenuated in some individuals. The hyobranchial apparatus of most *Cynops* was similar to that described for *C. pyrrhogaster* by Özeti and Wake (1969) with straight epibranchials. However, the epibranchials in some *C. cyanurus* are relatively straight, whereas others are moderately curved and those of *C. wolterstorffi* moderately to strongly curved (Özeti and Wake, 1969). Except for one individual with 14 trunk vertebrae, all *Cynops* had 13 trunk vertebrae and like *Paramesotriton* and *Pachytriton,* two or three caudo-sacral vertebrae.

There are osteological differences among the species of *Cynops,* which can be divided into four groups based on two main characters: the length of the frontal process of the premaxilla, and the degree of contact of the nasals (Fig. 4). *Cynops orphicus* is the only species in our sample with a long frontal process of the premaxilla and with nasals widely separated as in *Paramesotriton* and *Pachytriton.* In *Cynops cyanurus* and *Cynops wolterstorffi,* the frontal process of the premaxilla is long and the nasals almost or narrowly contact one another. The nasals of *Cynops orientalis* almost or narrowly contact one another as well, but the frontal process of the pre-

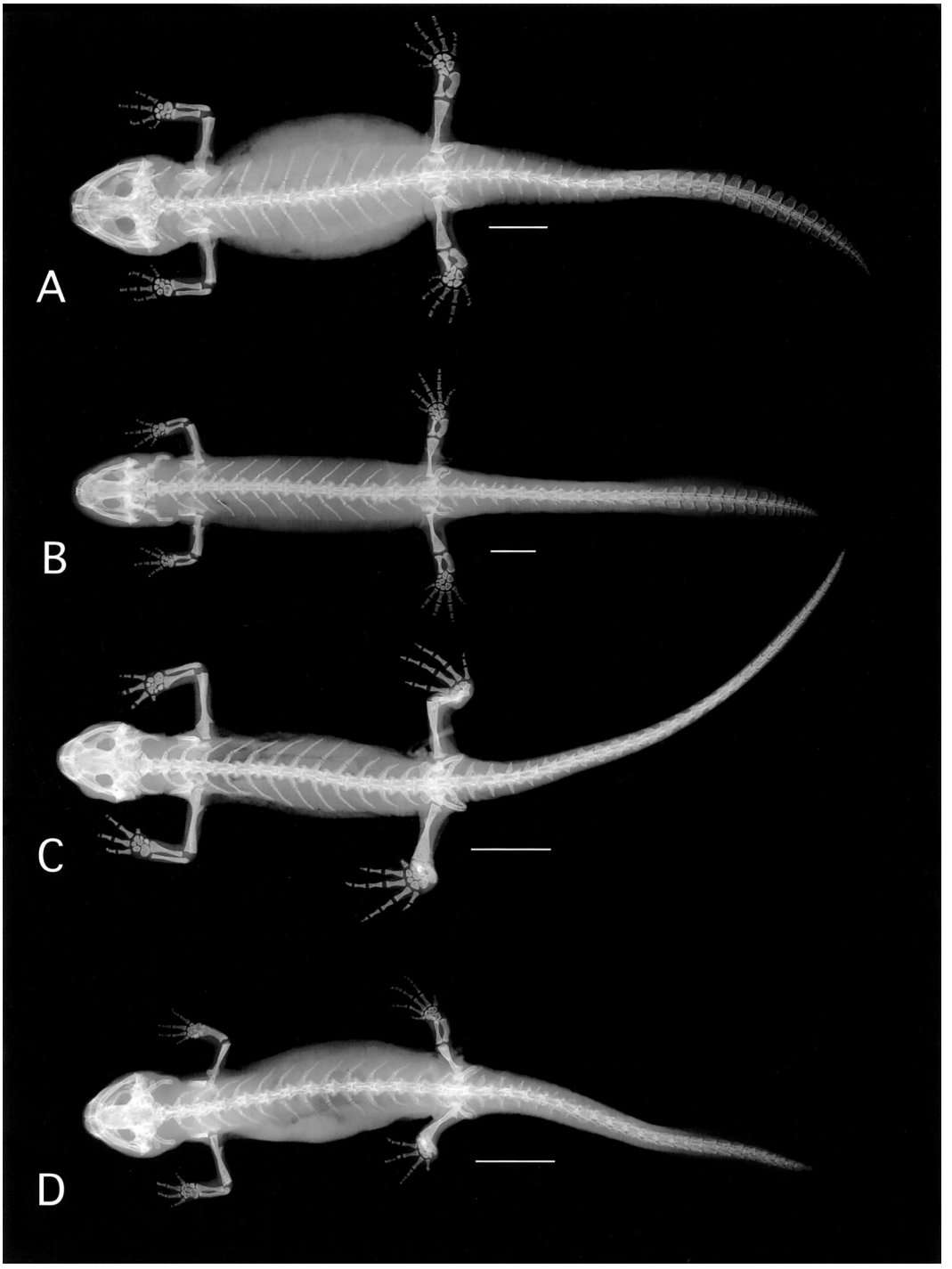

Fig. 3. X-rays of the three salamandrid genera used in this study. (A) *Paramesotriton hongkongensis* (MVZ 230370), (B) *Pachytriton labiatus* (MVZ 230358), (C) *Cynops pyrrhogaster* (MVZ 191972), (D) *Cynops cyanurus* (MVZ 219758). Scale bar under each individual equals one centimeter.

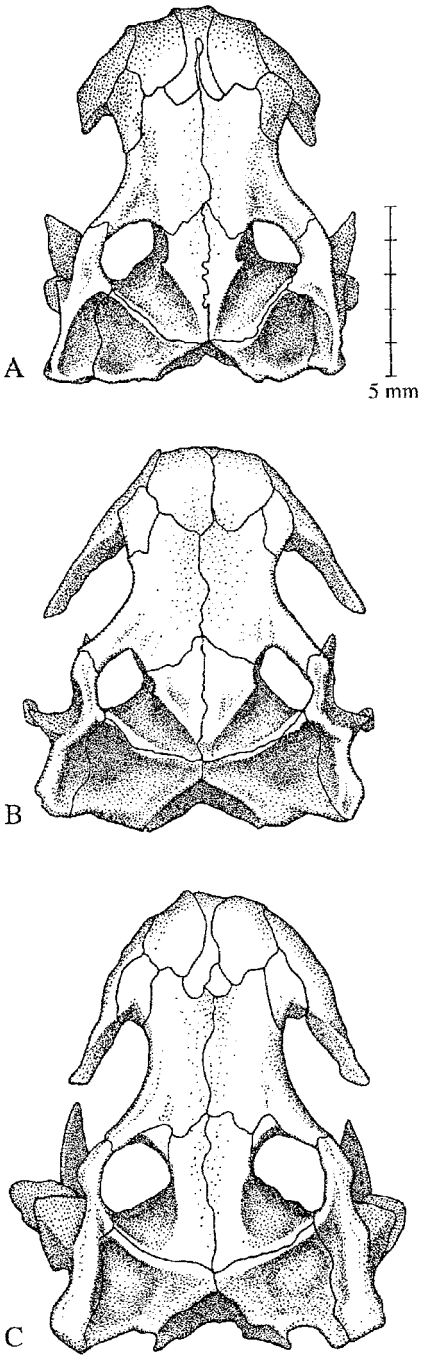

Fig. 4. Dorsal view of the skulls of representatives from three of the four *Cynops* species groups. (A) *Cynops orphicus* (MVZ 22465), (B) *Cynops pyrrhogaster* (MVZ 185212), and (C) *Cynops wolterstorffi* (AMNH 5455).

maxilla is short rather than long (Zhao and Hu, 1988). In *Cynops pyrrhogaster* and *Cynops ensicauda,* the frontal process of the premaxilla is short, but the nasals broadly contact one another.

Discussion

Morphological evidence for the monophyly of *Cynops, Paramesotriton,* and *Pachytriton* has been weak, and authors have had varied interpretations. Some studies based mainly on general morphology (e.g., Freytag, 1962) considered the three genera to be close relatives, whereas a character-based analysis of feeding morphology and other features (Wake and Özeti, 1969) failed to find strong evidence of monophyly.

More recently, evidence concerning the relationships has come from molecular studies. Hayashi and Matsui (1989) made the first attempt at discerning relationships among salamandrid genera with molecular data, examining allozymic variation at 17 loci in 11 species. Unfortunately, their sample did not include any *Pachytriton,* so we cannot infer from their cladogram the position of that genus. Nonetheless, they recovered a well-supported clade that included *Cynops* and *Paramesotriton,* with little resolution. Titus and Larson (1995) used mitochondrial sequences of 12S and 16S mtDNA and morphological characters to examine evolutionary relationships within the family. Their best-supported combined tree reveals strong support for the monophyly of the *Cynops, Paramesotriton,* and *Pachytriton* clade (100 bootstrap) but with no resolution among the species within it. In their topology, these genera are represented as a trichotomy, with one of the *Triturus* species as the sister taxon to this group. Despite differences in sampling and methodology, collectively these studies suggest the three genera are probably each others' closest relatives.

The seven species of *Cynops* cluster into three well-defined species groups (Zhao and Hu, 1988): the *pyrrhogaster* group (including *pyrrhogaster* and *ensicauda*), the monotypic *orientalis* group, and the *wolterstorffi* group (including both *wolterstorffi* and *cyanurus*). Zhao and Hu (1988) considered the *pyrrhogaster* species group to be the most basal of the three based on morphological and behavioral characters. In the molecular portion of this study, we included representatives from the *pyrrhogaster* and *wolterstorffi* species groups. Our topology supports the paraphyly of this genus and suggests that *C. pyrrhogaster* may be more closely related to *Paramesotriton* and *Pachytriton* than to *C. cyanurus.*

Wake and Özeti (1969) proposed that *Cynops* had a more ''generalized'' morphology, and one might expect that more specialized or derived morphologies would evolve from within a group such as this one.

We cannot say with certainty that the two members of the genus *Cynops* are a paraphyletic assemblage from the molecular data alone because tree comparison tests do not reject the possibility that this genus is monophyletic. However, distinct cranial morphologies among *Cynops* species groups underscore the possibility that *Cynops* may be paraphyletic and that both *Pachytriton* and *Paramesotriton* may have evolved from within *Cynops* as currently recognized. *Cynops* can be divided into four groups on the basis of two cranial characters. Broad versus narrow contact of the nasals and long versus short frontal process of the premaxilla together distinguish the *pyrrhogaster* group, *wolterstorffi* group, *orientalis* group, and *C. orphicus*. The first three species groups were previously defined by Zhao and Hu (1988), and we suggest that based on cranial variation alone, *C. orphicus* could be considered a separate monotypic *orphicus* group. We were unable to examine any specimen of *C. chenggongensis,* but assignment of this species to one of these morphological groups should be possible with the examination of these characters.

Although not analyzed in a cladistic framework, the data presented by Zhao and Hu (1988) showed that *Paramesotriton* and *Pachytriton* share many derived character states for osteological and hyoid apparatus characters relative to *Cynops*. Our topology supports this relationship; the *Paramesotriton* and *Pachytriton* in our study represent a monophyletic assemblage relatively well supported by our analyses (bootstrap values of 57–62%).

The *Pachytriton* clade in our topology is well supported (100 bootstrap, 16 decay). We recorded relatively large amounts of divergence (K2p of 0.3–3.5%) within what is currently considered to be a single species, *P. labiatus,* and our results suggest that more than a single species may be represented. Thiesmeier and Hornberg (1997) discussed two undescribed species, and a complete revision of this genus is in order. *Pachytriton* appears to have a more derived morphology, possibly resulting from adaptation to an almost completely aquatic life in fast-moving streams (Pope and Boring, 1940); they have smooth skin, a narrow skull, and an uncompressed tail. Additionally, the hyobranchial apparatus of *Pachytriton* is highly specialized for aquatic ''gape and suck'' feeding, more than that of other salamandrid species (Özeti and Wake, 1969).

The support for a *Paramesotriton* clade including *guangxiensis, deloustali,* and *hongkongensis* is relatively high (96 bootstrap, 7 decay), but support for a clade including the fourth *Paramesotriton, P. caudopunctatus,* is low (31 bootstrap, 1 decay); thus, we cannot say with certainty that *Paramesotriton* is monophyletic. In contrast to other species of *Paramesotriton, P. caudopunctatus* is easily diagnosed on the basis of external morphology and coloration. Thus, we are confident that our single sample from a commercial specimen was correctly identified (T. Titus, pers. comm.). Nonetheless, given the observed genetic distances and the interesting position of this taxon on our tree future systematic studies should confirm our findings. Although not analyzed in a cladistic framework, morphological data are in agreement with our topology, with *P. guangxiensis, deloustali,* and *hongkongensis* more similar to one another than to *P. caudopunctatus*. Like other *Paramesotriton, P. caudopunctatus* has granular skin, a *Paramesotriton*-like skull (e.g., complete fronto-squamosal arch, maxillaries at an angle to pterygoid, nasals separated), and prominent vertebral, lateral, and parietal ridges, which suggest that it is allied with other *Paramesotriton*. However, the skull of *P. caudopunctatus* is long and narrow, and the epibranchials of the hyobranchial apparatus are flared like those of *Pachytriton*. Thus, the morphological data exhibit a combination of *Paramesotriton*-like and *Pachytriton*-like characters. This may be a result of the basal position of the species within the genus or it might also reflect the ecology of this species, which is stream dwelling compared to the other members of the genus which are predominantly pond dwellers (Bischoff and Böhme, 1980). Some behavioral characteristics of *P. caudopunctatus* are also *Pachytriton*-like; their courtship pattern resembles that of other *Paramesotriton,* whereas their egg-laying and feeding behaviors are similar to those of *Pachytriton* (Sparreboom, 1983; Rehák, 1984).

Relationships within *Paramesotriton* have not been examined in a detailed cladistic framework. Several species of *Paramesotriton* have been described only recently (Liu and Hu, 1973; Huang et al., 1983; Wen, 1989), and more forms likely will become known with further collection efforts in southeast Asia. In the original descriptions, authors noted gross morphological similarities between pairs of taxa and suggested their close relationship (e.g., Wen, 1989). A phenetic comparison of the morphology of five of the six species showed that *P. fuzhongensis* and

chinensis are very similar (Pang et al., 1992). In addition, Pang et al. concluded that *P. guangxiensis* and *hongkongensis* were close relatives and basal to *fuzhongensis, chinensis,* and *caudopunctatus*. Analysis of our molecular data finds *P. caudopunctatus* to be the most basal of the species of *Paramesotriton*. Tissue samples of *chinensis* and *fuzhongensis* were not available to us; however, external, cranial, and hyobranchial characteristics show these three species to be more similar to *guangxiensis, deloustali,* and *hongkongensis* than to *caudopunctatus*. Based on morphological evidence for all *Paramesotriton* examined and molecular evidence for four or the six species of *Paramesotriton,* we suggest that *chinensis* and *fuzhongensis* are more recently diverged than *P. caudopunctatus*.

Our molecular data show that *C. cyanurus* is deeply differentiated from all other samples (lowest K2p is 13.2% to *C. pyrrhogaster*) and that it is basal to the remainder of the Asian taxa examined here. We were not able to secure molecular sequences for *C. wolterstorffi* (which is likely extinct, E. Zhao and D. Yang, pers. comm.), but morphological features of the species ally it with *C. cyanurus*; both species are highland forms that live in the same general region. Should the paraphyly of *Cynops* be confirmed, an appropriate taxonomic resolution would be to recognize the genus *Hypselotriton* (Wolterstorff, 1934) as a valid taxon containing at least *cyanurus* and *wolterstorffi*.

Although this study contributes to our understanding of the relationships among species in this Asian salamander radiation, there are still many questions to be addressed. In general, studies of these taxa have been plagued by reduced number of characters available because of conserved morphology in this group or limited availability of multiple, reliable samples of all taxa. Molecular studies have clarified some of the relationships within this group (Hayashi and Matsui, 1989; Titus and Larson, 1995) but with only limited success. Given these limitations and the apparent paraphyletic nature of some currently recognized genera, we predict that future studies combining morphology and independent mitochondrial and nuclear markers will be most successful in elucidating relationships within this clade.

Morphological Material Examined

Cynops cyanurus. —MVZ 219757–219760.
Cynops ensicauda.—CAS 22598, MVZ 57903–57904.
Cynops orientalis.—MVZ 204305–204308.
Cynops orphicus.—MVZ 22460, 22468, 22472.
Cynops pyrrhogaster.—MVZ 22656–22657, 185212, 191972–191973, 198773–198774.
Cynops wolterstorffi.—AMNH 5453–5455, CAS 6664, 54852, 54908–54909, MCZ 7170, 7173, 8154–8157, 8751, 9621.
Pachytriton brevipes.—MVZ 204297, 204299–204300, 206174.
Pachytriton labiatus.—MVZ 230147, 230354–230359, MVZ 230720.
Pachytriton sp. nov. *B*.—MVZ 206173.
Paramesotriton caudopunctatus.—MVZ 204295–204296.
Paramesotriton chinensis.—CAS 6380, MVZ 230360.
Paramesotriton deloustali.—MVZ 206310–206312, 222122, 223627–223629, 225135, 226269–226270.
Paramesotriton fuzhongensis.—MVZ 230361–230364.
Paramesotriton guangxiensis.—MVZ 220905–220906.
Paramesotriton hongkongensis.—MVZ 110576–110578, 184859–184860, 198499, 198697–198698, 198700–198704, 219766, 230370.

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APPENDIX 1. ALL SPECIMENS OF SALAMANDRIDAE SEQUENCED FOR MOLECULAR ANALYSIS. The full taxon name, voucher number, and collection locality are listed for ingroup and outgroup taxa. Institutional abbreviations are as listed in Leviton et al. (1985).

Abbreviation	Taxon	Specimen #	Locality
Pdel1	*Paramesotriton deloustali*	MVZ 222122	Tam Dao, Vinh Phu Prov., Vietnam
Pdel2	*Paramesotriton deloustali*	MVZ 223628	Tam Dao, Vinh Phu Prov., Vietnam
Pdel3	*Paramesotriton deloustali*	MVZ 223629	Tam Dao, Vinh Phu Prov., Vietnam
Pdel4	*Paramesotriton deloustali*	MVZ 223627	Tam Dao, Vinh Phu Prov., Vietnam
Pdel5	*Paramesotriton deloustali*	UTA 40127	Tam Dao, Vinh Phu Prov., Vietnam
Pgua1	*Paramesotriton guangxiensis*	MVZ 220905	Linming Co., Guang Xi Prov., China
Pgua2	*Paramesotriton guangxiensis*	MVZ 220906	Linming Co., Guang Xi Prov., China
Phon4	*Paramesotriton hongkongensis*	MVZ 230366	Ho Chung Valley, Hong Kong Island, China
Phon7	*Paramesotriton hongkongensis*	MVZ 230369	Hong Kong Island, China
Pcau1	*Paramesotriton caudopunctatus*	no voucher	commercial specimen
Plab1	*Pachytriton labiatus*	MVZ 230720	commercial specimen
Plab2	*Pachytriton labiatus*	CAS 194298	Jiaxing Prefecture, Zhejiang Prov., China
Plab3	*Pachytriton labiatus*	MVZ 230147	commercial specimen
Cpyr1	*Cynops pyrrhogaster*	KU 219723	commercial specimen
Ccya1	*Cynops cyanurus*	MVZ 219757	Chuxiong, Yunnan Prov., China
Ccya2	*Cynops cyanurus*	MVZ 219758	Chuxiong, Yunnan Prov., China
Tgra1	*Taricha granulosa*	KU 219725	Camp Kilowan, Polk Co., Oregon
Ttal1	*Tylototriton taliangensis*	CAS 195126	Autonomous Prefecture, Sichuan Prov., China
Tver1	*Tylototriton verrucosus*	MVZ 219761	Jingdong, Yunnan Prov., China

Appendix 2. Pairwise Sequence Divergence among the 17 Individuals Included in the Molecular Analyses. Above diagonal: K2p corrected divergences. Below diagonal: absolute number of changes (bp, total length 786).

		1	2	3	4	5	6	7	8
1	Pdel2	—	0.002	0.087	0.081	0.104	0.101	0.128	0.133
2	Pdel3	1	—	0.088	0.083	0.106	0.103	0.130	0.135
3	Pgua1	52	53	—	0.002	0.121	0.118	0.158	0.133
4	Pgua2	50	51	1	—	0.115	0.113	0.154	0.129
5	Phon4	62	63	70	67	—	0.001	0.113	0.133
6	Phon7	61	62	69	67	1	—	0.114	0.128
7	Pcau1	73	74	87	85	71	69	—	0.109
8	Plab1	79	80	77	76	84	80	69	—
9	Plab2	80	81	79	76	82	80	66	2
10	Plab3	83	84	88	86	89	87	71	23
11	Cpyr1	82	83	85	82	89	88	69	74
12	Ccya2	92	93	102	100	105	101	94	91
13	Tvul1	114	114	112	110	118	115	115	117
14	Tcar1	104	104	108	106	113	109	113	113
15	Tgra1	146	147	148	149	153	153	148	159
16	Ttal1	146	147	144	143	151	146	149	152
17	Tver1	144	145	142	141	141	140	139	144

Appendix 2. Extended

		9	10	11	12	13	14	15	16	17
1	Pdel2	0.137	0.148	0.141	0.167	0.248	0.221	0.263	0.278	0.271
2	Pdel3	0.139	0.150	0.143	0.169	0.248	0.221	0.266	0.281	0.273
3	Pgua1	0.137	0.159	0.148	0.189	0.246	0.233	0.278	0.277	0.270
4	Pgua2	0.131	0.155	0.142	0.186	0.241	0.229	0.273	0.275	0.267
5	Phon4	0.133	0.147	0.142	0.177	0.232	0.219	0.262	0.264	0.248
6	Phon7	0.130	0.147	0.144	0.175	0.235	0.219	0.267	0.263	0.248
7	Pcau1	0.107	0.114	0.108	0.155	0.219	0.214	0.255	0.260	0.245
8	Plab1	0.003	0.035	0.115	0.149	0.226	0.216	0.267	0.268	0.256
9	Plab2	—	0.031	0.110	0.146	0.232	0.229	0.271	0.269	0.256
10	Plab3	20	—	0.125	0.156	0.243	0.232	0.257	0.273	0.256
11	Cpyr1	69	77	—	0.132	0.209	0.221	0.249	0.248	0.231
12	Ccya2	87	94	82	—	0.245	0.218	0.277	0.279	0.246
13	Tvul1	115	122	111	124	—	0.188	0.189	0.232	0.210
14	Tcar1	114	118	116	113	107	—	0.198	0.223	0.231
15	Tgra1	154	147	149	156	101	105	—	0.233	0.195
16	Ttal1	149	153	144	157	119	116	136	—	0.095
17	Tver1	144	143	133	139	107	116	115	59	—