

Phylogenetic relationships, taxonomy, character evolution and biogeography of the lacertid lizards of the genus *Takydromus* (Reptilia: Squamata): a molecular perspective

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Historical relationships were inferred for the oriental lizards of the genus *Takydromus* Daudin 1802 (Lacertidae) on the basis of DNA sequences. Of the 17 species currently recognized for the genus, 13 species represented by 42 specimens from 29 localities were examined. Maximum-likelihood and maximum-parsimony analyses of data for 829 aligned sites from parts of the mitochondrial 12S and 16S rRNA genes yielded relationships that, while showing no substantial discrepancy with each other, were strikingly different from a currently prevailing phylogenetic hypothesis from a parsimony analysis of morphological characters. Based on the results of these molecular analyses, supplemented by results of the morphological analysis that offered robust evidence for positions of two additional species (*T. khasiensis* and *T. sylvaticus*), the following interrelationships were hypothesized as the most preferred phylogeny: (*kuehnei* (*sexlineatus khasiensis*))(*tachydromoides* ((*smaragdinus (sauteri (dorsalis sylvaticus))*)) (*amurensis* (((*formosanus wolteri*) *hsuehshanensis*)(*toyamai (septentrionalis stejnegeri)*)))))). These interrelationships indicate: (1) invalidity of *Platyplacopus* Boulenger 1917, which was recently resurrected as a subgenus of *Takydromus* on the basis of the morphological analysis; (2) homoplasy in states of some morphological characters, such as green dorsal coloration, that were considered as synapomorphs of certain nodes in the morphological analysis; and (3) involvement of the genus in a series of vicariations in both the continental and insular parts of eastern Eurasia. Due to the paucity of available samples, phylogenetic status of *T. intermedius* and *T. haughtonianus* remain to be examined in future studies. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 76, 493–509.

ADDITIONAL KEYWORDS: dispersal – eastern Eurasia – homoplasy – maximum-likelihood analysis – maximum-parsimony analysis – mitochondrial DNA – palaeogeography – ribosomal RNA genes – subgeneric classification – vicariance.

INTRODUCTION

The genus *Takydromus* Daudin 1802 is an eastern Eurasian representative of the family Lacertidae

(Reptilia: Squamata), which is otherwise almost exclusively diversified in western Eurasia and Africa (Arnold, 1989a; Fu, 1998; Harris, Arnold & Thomas, 1998a; Harris, 1999). Arnold (1997) recognized 16–17 species for this genus, hypothesizing historical relationships for them (with the exception of the enigmatic

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T. haughtonianus) on the basis of parsimony analysis of 35 morphological characters (Appendix 1). However, his analysis left some relationships unresolved, while many others failed to receive substantial bootstrap support during the present evaluation (Fig. 1). Considering the remarkable morphological plasticity exhibited by other lacertid genera (e.g. Arnold, 1989b, 1998; Harris, Arnold & Thomas, 1998b; Harris & Arnold, 2000), such poor bootstrap support for most nodes in the morphological tree may reflect frequent occurrence of homoplasy, although it may be due simply to his using insufficient characters for the analysis.

Takydromus contains extensive morphological and ecological variation (e.g. Takenaka, 1989; Arnold, 1997; Huang, 1998), and thus might be expected, like

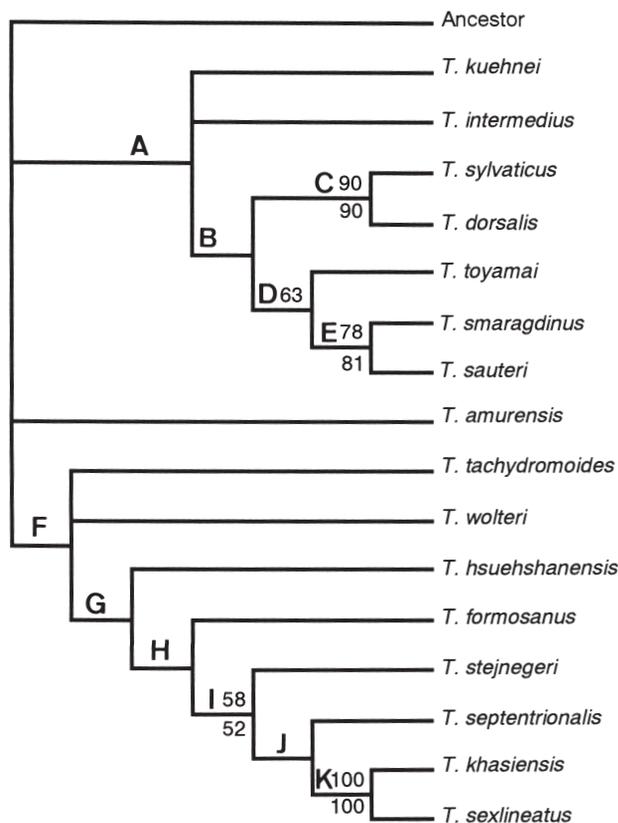


Figure 1. Strict consensus tree of six equally parsimonious cladograms derived from the analyses of morphological data. Numerals above and below nodes denote percentages of supports in 1000 bootstrap proportions (BPs: Swofford, 1998) for the original data set in Arnold (1997), and for the data set supplemented by results of morphological examinations by us, respectively. Branches without BP values were not supported in $\geq 50\%$ of the replicates. Letters at nodes correspond to those in Arnold (1997).

other lacertid genera, to provide a useful example for studying the patterns and processes of evolution in relevant traits (e.g. Bauwens *et al.*, 1995; Bauwens & Diaz-Uriarte, 1997). It also appears to be ideal material for investigating the evolutionary consequences of past geological events as expressed in a phylogeographical pattern of extant taxa, because, unlike almost all other lineages of non-volant terrestrial vertebrates in eastern Eurasia, it is extant throughout the continent and islands in this region (Table 1, Fig. 2) with a complex geological history (e.g. see Kizaki & Oshiro, 1980 and Ota, 1998 for review). The lack of robust phylogenetic hypothesis for the genus, however, makes it difficult to take full advantage of this.

Several recent studies have suggested that molecular analysis is particularly effective for elucidating historical interrelationships among lacertid lizards that might otherwise be obscured by extensive parallelism, reversal, and convergence in morphological evolution (Fu, 1998; Harris *et al.*, 1998a,b; Harris, 1999; Harris & Arnold, 2000; but see Fu, 2000 for discussion of practical problems in applying this to the higher category phylogeny of the Lacertidae). In this study, we infer phylogenetic relationships within *Takydromus* on the basis of analyses of sequence variations in mitochondrial DNA (mtDNA). We also discuss the implications of the resulting phylogeny for the subgeneric classification, character evolution, and historical biogeography of the genus.

MATERIAL AND METHODS

SAMPLES EXAMINED AND METHODS FOR DNA SEQUENCING

Tissues were obtained from 42 specimens representing 29 local populations of 13 species of *Takydromus* (Appendix 2). These include representatives of both subgenera, *Takydromus* and *Platyplacops*, proposed by Arnold (1997). We used two species of other lacertid genera *Lacerta* (*L. vivipara*) and *Podarcis* (*P. muralis*), as outgroups for the phylogenetic analysis, as recent studies indicated that these genera constitute a monophyletic group within the Lacertidae, together with *Takydromus* and a few other genera (Harris, 1999; Fu, 2000). All voucher specimens were deposited in the zoological collection of the Kyoto University Museum (KUZ; see Appendix 2).

The methods of DNA extraction, amplification and sequencing are described in detail elsewhere (Honda *et al.*, 1999a,b). Part of the mitochondrial 12S and 16S ribosomal RNA (rRNA) genes were amplified by PCR (Saiki *et al.*, 1988); the 12S rRNA primers were L1091 (5'-AAACTGGGATTAGATACCCCACTAT-3') and H1478 (5'-GAGGGTGACGGGCGGTGTGT-3') (Kocher

Table 1. Geographic distribution of species of the genus *Takydromus*

Species	Distribution	Source
<i>T. kuehnei kuehnei</i>	Taiwan, Hainan, SE continental China	Arnold (1997)
<i>T. k. vietnamensis</i>	N Vietnam	Ziegler & Bischoff (1999)
<i>T. s. sexlineatus</i>	W Indochina, N Malaya, Sunda-shelf Islands	Arnold (1997)
<i>T. s. ocellatus</i>	E Indochina, N Malaya, Hainan S continental China	Arnold (1997)
<i>T. khasiensis</i>	NE India	Arnold (1997)
<i>T. tachydromoides</i> *	Main islands & adjacent islets of Japan	Nakamura & Ueno (1963)
<i>T. smaragdinus</i> *	C Ryukyus, Japan	Takeda & Ota (1996)
<i>T. sauteri</i> *	S Taiwan & SE off-shore islet	Lue, Tu & Shang (1999)
<i>T. dorsalis</i> *	Yaeyama Islands (S Ryukyus)	Arnold (1997)
<i>T. sylvaticus</i>	E continental China	Arnold (1997)
<i>T. amurensis</i>	E Russia, Korea, NE continental China, Tsushima	Arnold (1997)
<i>T. formosanus</i> *	Taiwan & eastern offshore-islet	Lue <i>et al.</i> (1999)
<i>T. wolteri</i>	E Russia, E continental China, Korea including Cheju	Arnold (1997)
<i>T. hsuehshanensis</i> *	Taiwan (high altitude areas only)	Lue <i>et al.</i> (1999)
<i>T. toyamai</i> *	Miyako Islands (S Ryukyus)	Takeda & Ota (1996)
<i>T. septentrionalis</i>	E continental China & eastern off-shore islets	Arnold (1997), Ota & Lin (1997)
<i>T. stejnegeri</i> *	Taiwan & western off-shore islets	Lue <i>et al.</i> (1999)
<i>T. intermedius</i>	SW & S continental China	Arnold (1997)
<i>T. haughtonianus</i>	NE India	Arnold (1997)

*Species endemic to the East Asian landbridge islands.

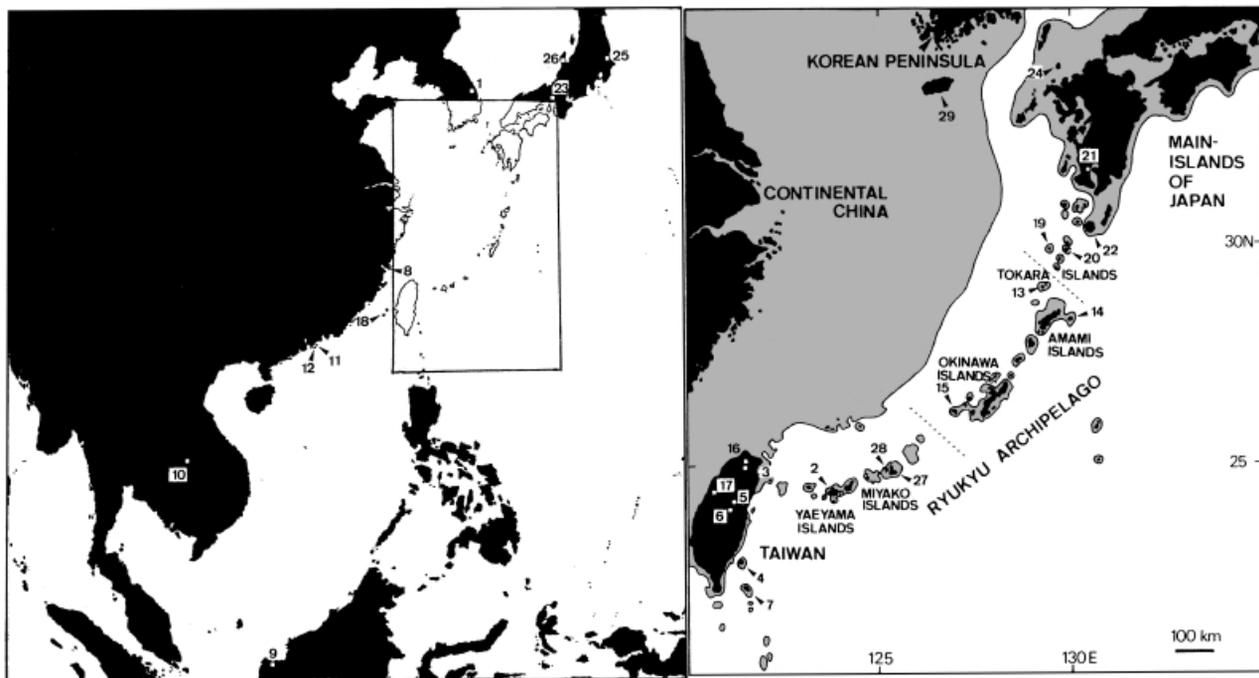


Figure 2. Map of East Asia (left) and magnification of its inset section (right). Numerals denote localities of examined specimens listed in Appendix-2. Stippled portions, and dashed lines in the latter indicate, respectively, putative additional land areas that should have been exposed above sea level during the late Pleistocene glaciation (15 000–20 000 year BP) (currently under the sea but up to 120 m deep), and locations of the Tokara Tectonic Strait and the Kerama Gap. See Ota *et al.* (1993) and Ota (1998) for the background theory and method for the inference of past land configurations around the Ryukyu Archipelago in detail.

et al., 1989), and the 16S rRNA primers were L2606 (5'-CTGACCGTGCAAAGGTAGCGTAATCACT-3') and H3056 (5'-CTCCGGTCTGAACTCAGATCACGTAGG-3')(Hedges, Nussbaum & Maxon, 1993). The numbering system followed that for the human sequence (Anderson *et al.*, 1981).

MORPHOLOGICAL INVESTIGATION

We also examined states of the 35 morphological characters described by Arnold (1997) for those specimens from which tissues were taken for molecular analysis (Appendix 2). For *T. toyamai* and *T. sauteri*, characters of hemipenial morphology (nos. 33–35, and 33–34, respectively: see Appendix 1) were examined for the first time.

PHYLOGENETIC ANALYSIS

Alignments for DNA sequences were determined based on maximum nucleotide similarity using CLUSTAL X 1.8 (Thompson *et al.*, 1994) with default gap penalties. The output was then adjusted by eye using manual aligner SeqApp 1.9 (Gilbert, 1993).

Using PAUP* 4.0b (Swofford, 1998), the partition homogeneity test (Farris *et al.*, 1994) was applied to test the compatibility among data sets with 1000 heuristic replicates, while the Templeton and two-tailed Wilcoxon signed rank tests (Templeton, 1983; Felsenstein, 1985b), were applied to evaluate the statistical significance of the shortest tree generated from morphological (Appendix 1) and DNA sequence (Appendix 3) data sets.

The maximum-likelihood (ML) and maximum-parsimony (MP) analyses (the latter using the heuristic search option) were also performed using PAUP. The empirical base frequencies and equal rate substitution model was adopted. No transition-transversion bias was assumed. The confidence of branches was assessed by bootstrap resamplings (Felsenstein, 1985a) in both of the analyses.

The interpretation of bootstrap proportions (BPs) is still in a state of uncertainty (see Felsenstein & Kishino, 1993; Hillis & Bull, 1993). We tentatively followed Shaffer *et al.* (1997), and considered BPs of $\geq 90\%$ to be highly significant, 70–90% to be marginally significant, and 50–70% as constituting limited evidence of monophyly.

RESULTS

MORPHOLOGICAL CHARACTERS

Morphological examination of our specimens, while yielding results not contradicting those of Arnold (1997), uncovered states of hemipenial characters in

T. toyamai and *T. sauteri* not determined during that study (Appendix 1). Hemipenes of both species shared general features with other congeneric hemipenes as described by Arnold (1986), and transverse sections of the lobes in retracted state were crescentic, not triskelial. In the hemipenis of *T. sauteri*, sulci extend into the lobes, while the outer lips of the lobe sulci become basally directed fleshy flaps. Such states were not recognized in the hemipenis of *T. toyamai* (Appendix 1).

MITOCHONDRIAL SEQUENCE VARIATION

Aligned sequences from 12S and 16S rRNA genes are presented in Appendix 3. All showed a distinct bias for adenine and against guanine on the light strand (A = 33.1–36.3%, C = 20.0–23.7%, G = 17.5–19.4%, T = 24.1–26.9%), closer to that of the genome than that of the nuclear integrated copies of mitochondrial genes (Zhang & Hewitt, 1996; Macey *et al.*, 1997). We interpreted them as being those of authentic mtDNA.

In the sequence data, transitions exceeded transversions at low levels of divergence. This agrees with previous studies on animal mtDNA that have reported an initially high (>50%) transition bias gradually decreasing over time (Brown *et al.*, 1982; Hedges, Bezy & Maxon, 1991; Fuller, Baverstock & King, 1998; Honda *et al.*, 2000a,b). The scatter plots did not exhibit a distinct transition plateau (not shown); this, usually corresponding to transitions of between 40% and 50%, is the point at which multiple substitutions occur at the same site (Thomas *et al.*, 1989; Hedges *et al.*, 1991). Therefore, the data were considered to be useful for phylogenetic inference (e.g. Fuller *et al.*, 1998; Honda *et al.*, 2000a,b; Yamagishi *et al.*, 2001).

The 12S rRNA fragment consisted of 395 total aligned sites, 147 of which were variable among the samples examined. For the 16S rRNA fragment, there were 434 total aligned sites, 171 of which were variable. Of the latter, one domain corresponding to nine successive sites in the aligned sequence was recognized as a broad gap in all *Takydromus* species except for *T. kuehnei* and *T. sexlineatus* (Appendix 3). Sequence data for this domain, as well as for other sites showing insertions or deletions in one or more samples, were excluded from the phylogenetic analysis.

The uncorrected p distance value in combined 12S and 16S rRNA sequences, calculated by the corresponding option of PAUP*4.0b (Swofford, 1998), was 0.122 between *L. vivipara* and *P. muralis*, whereas the values between *Takydromus* and the two outgroup taxa were 0.139–0.179 and 0.122–0.171, respectively. In pairwise comparisons of the ingroup species, the distance value varied from 0.028 (*septentrionalis* vs. *stejnegeri*) to 0.145 (*sexlineatus* vs. *tachydromoides*),

whereas that between the two conspecific subspecies, *T. sexlineatus sexlineatus* and *T. s. ocellatus*, was between 0.048 (Sarawak: site #9 in Fig. 2; see Appendix 2] vs. Thailand [site #10] and 0.062 (Sarawak vs. Hong Kong [site #11]) (Fig. 2). The uncorrected p distance values between conspecific (and consubspecific) samples were as follows: 0 in *T. amurensis* and *T. dorsalis*; 0–0.001 in *T. septentrionalis*; 0.001 in *T. sauteri* and *T. toyamai*; 0.002 in *T. s. sexlineatus*; 0–0.004 in *T. stejnegeri*; 0.006 in *T. hsuehshanensis*; 0.002–0.023 in *T. smaragdinus*; 0–0.025 in *T. s. ocellatus*; 0–0.034 in *T. formosanus*; 0.002–0.041 in *T. tachydromoides*. Of these, the greatest value (0.041) was observed between samples of *T. tachydromoides* from Nakanoshima, the Tokara Islands [site #19], and from Kagoshima, Kyushu, Japan [site #21].

PHYLOGENETIC RELATIONSHIPS

The partition homogeneity test revealed no significant heterogeneity between 12S and 16S rRNA sequences. We thus combined these two data sets in the following analysis. In contrast, the test revealed a remarkable heterogeneity between morphological (Appendix 1) and DNA sequence data sets (Appendix 3) ($P < 0.0001$). The morphological data set, derived from that of Arnold (1997), supplemented by our examination of voucher specimens (see above), was subjected to the MP analysis separately.

The ML phylogram derived from the aligned sequence data is shown in Figure 3. This tree, with largely significant BPs, provides detailed solution of infrageneric relationships as predicted by Fu (2000). Monophyly of the ingroup taxa (node 1) was supported by 100% BP. The ingroup was divided into two major lineages, of which one (node 2), consisting of *T. kuehnei* and *T. sexlineatus*, was supported by highly significant BP (93%). The other major lineage (node 3) received limited BP support (53%). *T. tachydromoides* split at the basal portion, and the remainder (node 4), also receiving limited BP support (55%), was divided into two branches, one (node 5) consisting of *T. smaragdinus*, *T. sauteri* and *T. dorsalis*, and the other (node 6) of the remaining six species. From the former assemblage that failed to receive substantial BP support (i.e. <50%), *T. smaragdinus* split first, leaving *T. sauteri* and *T. dorsalis* as closest to each other (node 7: BP = 72%), whereas in the latter (BP = 87%), *T. amurensis* split first and the remainder (node 8: BP = 100%) was divided into two subbranches, one (node 9: BP = 81%) consisting of *T. hsuehshanensis*, *T. wolteri* and *T. formosanus*, and the other (node 10: BP = 100%) of *T. toyamai*, *T. septentrionalis* and *T. stejnegeri*. In the former subbranch, *T. wolteri* and *T. formosanus* are suggested as constituting a monophyletic group (node 11) exclusive of *T. hsuehshanensis* but

with limited BP support (69%). In the latter subbranch, *T. toyamai* split first, leaving *T. stejnegeri* and *T. septentrionalis* as possible closest relatives to each other (node 12: BP = 64%).

Monophyly of conspecific (and consubspecific) samples received 99–100% BP support except for the *T. formosanus* samples from Hapen (Figs 2,3) and Lutao [site #4 in Fig. 2] (BP = 73%), and the *T. sexlineatus ocellatus* samples from Hong Kong area (Hong Kong Island [site #11] and Lantau Island [site #12]) and Thailand [site #10] (BP = 76%). Samples of *T. tachydromoides*, while receiving 100% BP support for monophyly as a whole, exhibited a prominent separation into two groups, one consisting of samples from islands south of Kyushu [sites #2, 19 and 20] (BP = 97%), and the other of samples from a much broader area of the main-islands of Japan [sites #21, 23, 25, 26] and an islet north of Kyushu [site #24] (BP = 71%).

The MP analysis yielded 20 equally parsimonious cladograms (834 steps; 256bp informative under the condition of parsimony; consistency index excluding uninformative characters = 0.54; retention index = 0.83; rescaled consistency index = 0.46). Relationships depicted in the strict consensus tree of these cladograms showed no discrepancy with those expressed in the ML phylogram (not shown). The MP analysis of the morphological data provided by Arnold (1997) with the addition of the hemipenial morphology data for *T. toyamai* and *T. sauteri* yielded a strict consensus cladogram which is identical with his cladogram in branching topology (see Fig. 1). Nodes between *T. khasiensis* and *T. sexlineatus*, and between *T. sylvaticus* and *T. dorsalis*, supported, respectively, with high (100%) and highly significant (90%) BPs by Arnold (1997), retained strong BP support in our analysis. On the other hand, the monophyly of *T. toyamai* with *T. smaragdinus* and *T. sauteri*, which originally received limited BP support (63%), was not supported by a substantial BP (i.e. <50%).

When the Templeton test was applied to the morphological data set, the topology in the MP cladogram was significantly more parsimonious than that from the molecular data set ($P < 0.001$). Likewise, when applied to the molecular data set, the MP cladogram was also significantly shorter than that from the morphological data set ($P < 0.0001$).

DISCUSSION

PREFERRED PHYLOGENY OF *TAKYDROMUS*

Several recent authors have argued that in the Lacertidae phylogenetic relationships estimated on the basis of molecular data more or less corroborate those hypothesized from morphological data at both

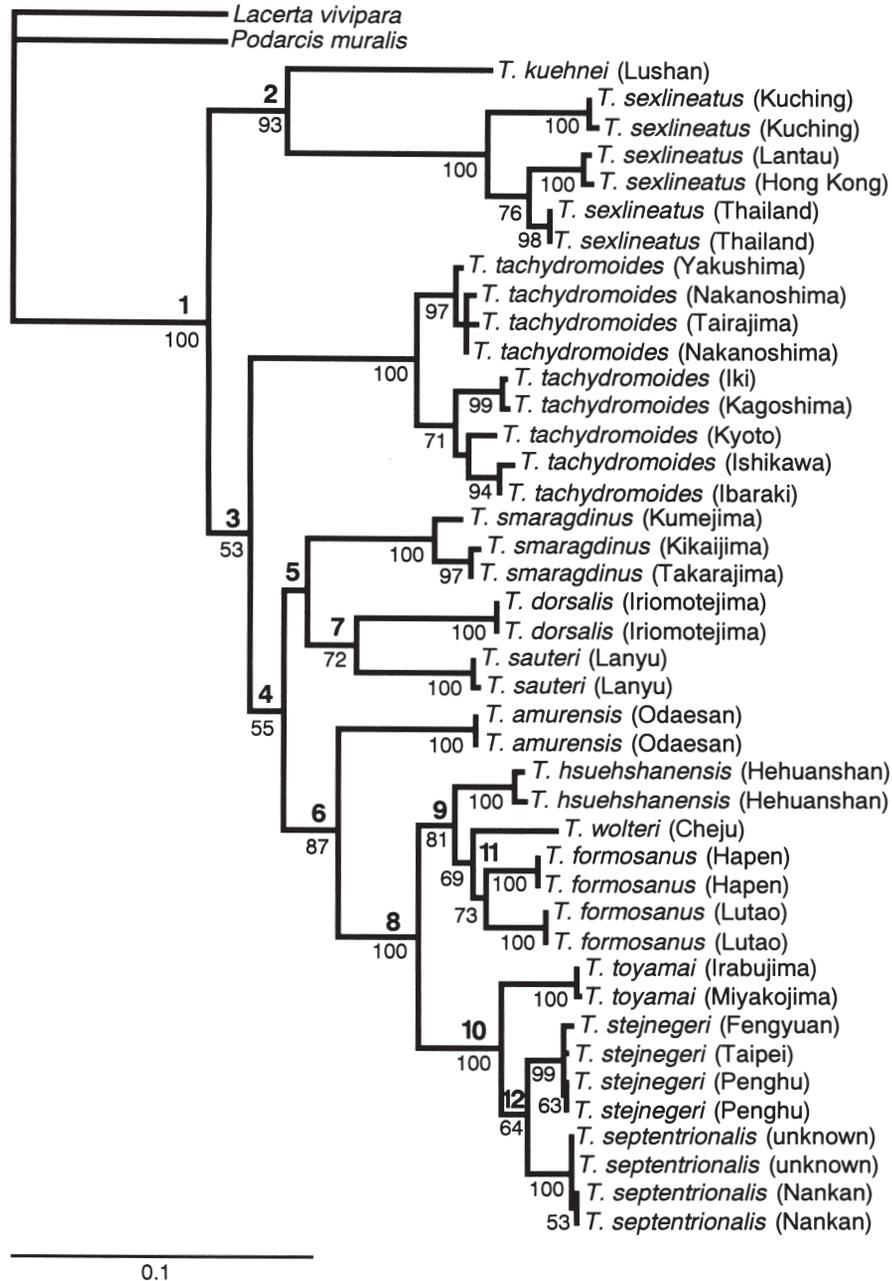


Figure 3. Maximum-likelihood (ML) phylogram for *Takydromus* samples derived from partial sequences of mitochondrial 12S and 16S rRNA genes (ln likelihood = -5729.6). Numerals above nodes are referred to in text. Those below nodes denote BP values supporting them. Branches without BP values were not supported in $\geq 50\%$ of the 100 replicates.

intergeneric (Harris *et al.*, 1998a; Harris, 1999) and intrageneric levels (Harris *et al.*, 1998b; Harris & Arnold, 2000, but see Fu, 1998 and below). With respect to *Takydromus*, however, analysis of mtDNA data yielded results (Fig. 3) that are fundamentally different from morphologically estimated interrelationships (Fig. 1). The Templeton test demonstrated a remarkable heterogeneity in phylogenetic structure

between morphological and DNA sequence data sets (see above). We are therefore left with the problem of devising the most preferable phylogenetic hypothesis.

Some authors advocate, in such cases, analysis of a combined data set (e.g. Eernisse & Kluge, 1993). A tree obtained by such a procedure, referred to as a combined or total evidence tree, usually corroborates the tree constructed solely on the basis of molecular data

due to the fact that the DNA sequence provides an overwhelmingly larger number of characters (e.g. Eernisse & Kluge, 1993; Harris & Arnold, 2000). Indeed, MP analysis of a combined data set from *Takydromus* species also yielded a tree that shows almost identical ingroup relationships with the molecular tree, but with much lower BPs at most nodes (not shown). Other authors, however, argue for the use of molecular data alone for the phylogenetic reconstruction, as long as they are available. This is based on the assumption that morphological characters are usually subjected to substantial selection forces from varying environments, whereas most DNA bases are much more neutral in substitution (e.g. Hedges & Maxon, 1996).

We believe that the combined tree is a tenable approach in that it makes the most objective use of available data. It is obvious, however, that this method seriously suffers when homoplasy prevails in a particular subset of data, because the principle of parsimony does not consider extensive occurrences of homoplasy in character evolution. Examination of the transition/transversion ratio in base substitutions between our samples provided no evidence of saturation (see above). Considering this, together with the prominent morphological plasticity in lacertid lizards implied by frequent morphological changes under varying environments (e.g. Arnold, 1989a,b, 1998; Harris *et al.*, 1998b), the highly significant heterogeneity between the morphological and mtDNA data sets (as well as considerable incongruence in topology between the morphological and the molecular trees) appears to reflect the prevalence of homoplasy in morphological characters in the process of diversification of *Takydromus*. It therefore seems appropriate to us in this instance to construct a phylogenetic hypothesis of the genus on the basis of mtDNA data alone (Hedges & Maxon, 1996).

As mentioned above, the ML phylogram (Fig. 3) provided a detailed solution, with largely significant BP support, for the interspecific relationships of *Takydromus*. The strict consensus cladogram from the MP analysis, though showing mostly lower BP, exhibited no discrepancy with the ML tree in topology (not shown). We thus consider the topology of the ML tree as representing most preferable phylogenetic hypothesis for the genus *Takydromus*. On the other hand, two of the species that were not examined in this study, *T. khasiensis* and *T. sylvaticus*, were indicated, respectively, to be sister taxa of *T. sexlineatus* and *T. dorsalis* with 100% and 90% BP support in the morphological analysis (Fig. 1). Character states supporting these relationships on the morphological tree (nos. 3[1], 6[1], 10[1], 12[1], 13[1], 14[1], 34[1] and 35[1] for the *T. khasiensis*–*T. sexlineatus* clade and nos. 17 [0:reversal] and 18 [0:reversal] for the *T. sylvaticus*–

T. dorsalis clade: see Arnold, 1997 and Appendix 1) showed no apparent contradictions in their phylogenetic significances when these relationships were added to the ML tree, although 3[1], 10[1] and 34[1] actually support the *T. khasiensis*–*T. sexlineatus*–*T. kuehnei* clade, rather than a clade of the former two, after such a modification. Moreover, when added to the ML tree, the two clades received additional support (nos. 15[1], 16[1], 18[2], 20[1], 23[1] and 31[1] for the former; and nos. 4[1], 22 [0:reversal] and 30 [0:reversal] for the latter). We thus tentatively add *T. khasiensis* and *T. sylvaticus* to relationships otherwise represented by the ML tree as sister species of *T. sexlineatus* and *T. dorsalis*, respectively.

Preferred interspecific relationships of *Takydromus* obtained through the above procedure are depicted in Figure 4. Of these, nodes 1, 2, 8 and 10 received 90% < BP support on the ML tree. We regard these relationships as most likely. Of the remaining nodes, nodes 6, 7 and 9 were marginally supported on the ML

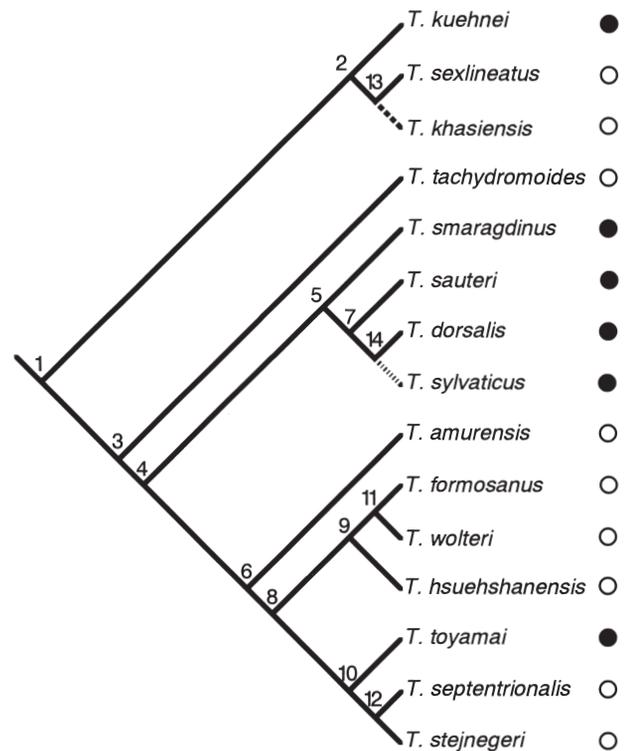


Figure 4. Preferred phylogenetic hypotheses for 15 *Takydromus* species. Numerals 1–12 above nodes correspond to those in Fig. 3. Dashed line and dotted line, both connecting species for which molecular data are not available, respectively, represent relationships supported by the analysis of the morphological data set with 100% and 90% BPs (see Fig. 1). Open and closed circles denote species that were assigned to the nominotypical subgenus and the subgenus *Platyplacopus* by Arnold (1997), respectively.

tree, and we consider all these relationships as moderately likely. Nodes 3, 4, 11 and 12 received limited support. We thus regard these relationships as fairly likely but needing careful verification on the basis of additional data and analysis. Node 5 failed to receive substantial BP support. As mentioned above, nodes 13 and 14 are supported only by morphological evidence, and verification of their validity by molecular analysis is urgently required. Due to the paucity of available samples, the phylogenetic status of *T. intermedius* and *T. haughtonianus* remains an open question.

TAXONOMIC IMPLICATIONS

Arnold (1997) resurrected *Platyplacopus*, a name originally published by Boulenger (1917) as a new full genus for *Takydromus kuehnei*. This was synonymized to *Takydromus* by Arnold (1989a) as a subgenus of *Takydromus* to accommodate several species (marked ● in Fig. 4 plus *T. intermedius*) constituting one of the major clades in his morphological cladogram (node A in Fig. 1). Our results, however, do not support this subgeneric classification, because they indicate non-monophyly of the subgenus *Platyplacopus* or of the nominotypical subgenus in Arnold's arrangement (marked ○ in Fig. 4). Instead, our results indicate a basal dichotomy of the genus into two major clades, one consisting of *T. kuehnei*, *T. sexlineatus* and *T. khasiensis*, and the other of the remaining species exclusive of the undetermined *T. intermedius* and *T. haughtonianus* (Fig. 4). This suggests that these relationships might be expressed by a new subgeneric classification.

In our cladogram, type species of both *Takydromus* (*T. sexlineatus*) and *Platyplacopus* (*T. kuehnei*) are assigned to the same major clade. The oldest generic name applicable to the other clade (node 3) is *Tachysaurus* Gray 1845, whose type species is *T. tachydromoides*. Even so, our analysis provided relatively weak BP support to node 3 (see above). Moreover, morphological examination by Arnold (1997) and by us yielded only one character state explicitly diagnostic of the node 3 components (no. 3[0]: see Appendix 1). We thus consider subgeneric division of the genus *Takydromus* to be premature.

Based on an unpublished manuscript by René Bourret (a zoologist who conducted a comprehensive herpetological survey in Vietnam before WWII), Ziegler, Quang & Böhme (1998) pointed out a possible sympatry of *T. sexlineatus ocellatus* and the nominotypical subspecies in Vietnam, which suggests the status of *ocellatus* as a full species. Our comparisons of mtDNA sequences between the two subspecies yielded the uncorrected p distance of 0.048–0.062, which, in addition to being the largest value from

intraspecific comparisons, exceeds values in a few combinations of different full species (e.g. 0.028 between *T. septentrionalis* and *T. stejnegeri*). Although the number of nucleotide site replacements (and the distance value) alone does not constitute sufficiently firm evidence for taxonomic decision-making, our results seem to offer circumstantial support to the prediction by Ziegler *et al.* (1998) regarding the status of *T. sexlineatus ocellatus*.

CHARACTER EVOLUTION

Arnold (1997) mapped on his cladogram morphological characters which support the nodes recognized therein. However, fundamental changes in tree topology by our analysis led to an essentially different picture of character evolution in *Takydromus*. Of the characters mapped at node A (see Fig. 1), for example, nos. 1, 11, 21, 33 and 34 seem to have evolved independently into derived states in nodes 2 and 5, or 2, 5 and *T. toyamai* (Fig. 4). Likewise, the derived states of character no. 32 (green dorsal colour), considered to explicitly support node B in Arnold's cladogram (Fig. 1), proved to be a consequence of convergence between node 5 and *T. toyamai* (Fig. 4) as predicted by Takeda & Ota (1996). Character 31, which derived state is interpreted by Arnold (1997) as supporting node I (Fig. 1), is likely to have evolved independently in nodes 11 and 12 (Fig. 4). Contrary to this, the derived state of character 3, suspected to have evolved independently in node K and *T. kuehnei* by Arnold (Fig. 1), is most likely a synapomorphy of node 2 (Fig. 4).

BIOGEOGRAPHY

Several authors have hypothesized from geographical patterns of taxonomic diversity and results of phylogenetic analyses that a series of early divergences of the family Lacertidae took place in western Eurasia, and that the ancestral form of *Takydromus*, deriving from one of these divergences dispersed into eastern Eurasia (e.g. Arnold, 1989a; Fu, 1998; Harris, 1999). For the divergence within the genus, however, no comprehensive biogeographical hypotheses have hitherto been proposed.

A survey of geographical distribution of *Takydromus* (Table 1) shows that nearly half of the species (8/17) are endemic to the East Asian landbridge islands that range from Taiwan to Japan (Kizaki & Oshiro, 1980). On the continent, distribution ranges from north-eastern India (Assam) to the eastern coast of Eurasia, with a partial extension into South-east Asia by *T. sexlineatus* and *T. kuehnei vietnamensis*. We have constructed a historical biogeographical hypothesis to explain the current phylogeographical pattern of

Takydromus on the ground of vicariance as long as possible, but invoking assumptions of dispersals when necessary.

Our analysis shows that in *Takydromus*, the initial dichotomy occurred between the *T. kuehnei*–*T. sexlineatus*–*T. khasiensis* clade and the remainder. Because component species of the former and latter clades are chiefly found from South-east Asia to southern China and in the more northern areas of eastern Eurasia, respectively (Table 1), it is likely that the initial divergence of the genus took place through a latitudinal isolation. Partial range overlap between representatives of these major clades in south-eastern China (Table 1) may be attributable to dispersals subsequent to such a vicariance event. In node 2, vicariance seems to have occurred initially between the ancestors of *T. kuehnei* from southern China and of the remainder from South-east Asia, and then between those of *T. khasiensis* from north-eastern India and of *T. sexlineatus* from an area ranging from Indochina to the Sunda shelf islands.

Node 3 accommodates the majority of the species of the genus including all insular endemics. The initial divergence of this node into node 4 and *T. tachydromoides* may have been involved by the latest isolation, through the establishment during the Pliocene of the Tokara Tectonic Strait (Fig. 2), of the main islands of Japan from the remaining landbridge extending from south-eastern continental China to the central Ryukyus (henceforth referred to as the Taiwan-Ryukyu landbridge) (Kizaki & Oshiro, 1980; Hayashi & Matsui, 1988; Hikida, Ota & Tayama, 1992; Ota, 1998). Since then, the main islands of Japan are considered to have been connected to the continent only through the Korean Peninsula, although this connection may have been broken and re-established several times, especially during the Pleistocene glaciations (Kizaki & Oshiro, 1980; Oshima, 1990; Park, Kimura & Taira, 1996). Colder climatic conditions in the eastern continent during these glaciations (which seem to have restricted the movement of a number of reptiles to more southern areas; Hikida, 1982), may have played a crucial role in preventing *T. tachydromoides* from dispersing into the continent.

In node 5, the possible basal lineage (*T. smaragdinus*), as well as two of the three other component species, is endemic to the East Asian islands. By contrast, the basal lineage of node 6 (*T. amurensis*) is largely found in the north-eastern part of the continent. This suggests that the major divergence of node 4 into nodes 5 and 6 occurred in the form of vicariance between the insular and continental areas.

In node 5, the initial divergence between lineages leading to *T. smaragdinus* and node 7 may have occurred through the formation of the Kerama Gap

(Fig. 2) during the early Pleistocene (Hikida & Ota, 1997; Ota, 1998; Hikida & Motokawa, 1999; Toda *et al.*, 1999; Tu *et al.*, 2000), because ranges of the former and the latter are confined to areas north-east and south-west of the strait, respectively. Absence of representatives of node 7 in areas immediately south-west of the strait (the Miyako Islands: Fig. 2), and between the Yaeyama Islands (range of *T. dorsalis*: Fig. 2) and southern Taiwan (*T. sauteri*) may be a consequence of competition with the node 8 species, because one (*T. toyamai*) and two (*T. formosanus* and *T. stejnegeri*) components of node 8 occur in these areas with complete and almost complete absence of range overlap with *T. dorsalis* and *T. sauteri*, respectively (Lue & Lai, 1991; Takeda & Ota, 1996). Endemic occurrence of possible sister species of *T. dorsalis* (i.e. *T. sylvaticus*) in the eastern continental area may suggest a dispersal of the common ancestor of node 14 from the insular area, but this view needs verification through careful examination of the validity of this node (see above).

The geographic range of *T. amurensis* is located north of the ranges of most node 8 species, although *T. wolteri* and *T. septentrionalis* show substantial and slight range overlaps with the former in the continent, respectively (Zhao & Adler, 1993; Zhao, Zhao & Zhou, 1999). This may reflect the occurrence of an initial latitudinal divergence in node 6, which gave rise to lineages leading to *T. amurensis* and the common ancestor of node 8, and subsequent dispersal of one or both of these lineages into the other's range. Because of the great range overlap between component species of nodes 9 and 10, we could not formulate any vicariance scenario for the dichotomy of node 8.

It is generally considered that Taiwan has experienced dry-land connection with the eastern continent several times since its initial insularization in the Pliocene (Huang, 1984; Yang, 1991). During each connection–isolation event, a number of lineages of terrestrial organisms supposedly dispersed into Taiwan. These were then involved in vicariance events, leading to the increase of taxonomic diversity on this large island (Yu, 1995; Ota, 1997). Ota (1997), on the basis of this biogeographical view, interpreted a portion of Arnold's cladogram – *hsuehshanensis* (*formosanus* (*stejnegeri* (*septentrionalis* (*khasiensis* *sexlineatus*)))) – as reflecting the dispersal–vicariance iteration in a single clade. However, our results suggest that the vicariance occurred independently in the *T. formosanus* (Taiwan)–*T. wolteri* (continent) clade, and the *T. stejnegeri* (Taiwan)–*T. septentrionalis* (continent) clade. For the divergence of *T. hsuehshanensis* (a species endemic to the high altitude areas of Taiwan) from node 11, one may assume a vicariance between the Taiwanese and continental ancestral

populations. However, derivation of *T. hsuehshanensis* from node 11 through an *in-situ* speciation is also likely, because, due to its remarkable diversity in geomorphology and climate, Taiwan has, it is thought, been acting as a 'stage of speciation' for a number of terrestrial vertebrate lineages (e.g. Yu, 1995; Ota, 1997; unpublished data; Ota, Ito & Lin, 1999). The former scenario necessitates an additional assumption for the subsequent dispersal of node 11 to Taiwan, whereas the latter scenario does not need such an assumption. We thus consider the *in-situ* divergence hypothesis more likely.

Dichotomy between *T. toyamai* from the Miyako Islands (Fig. 2) and node 12 species from Taiwan and the continent seems to be a consequence of the break-up of the Taiwan-southern Ryukyu landbridge sometime in the middle Pleistocene (Ota, 1998). The complete absence of this or related species in the Yaeyama Islands (Fig. 2) may be attributable to competition with *T. dorsalis*.

Sequence divergence of mtDNA at the infraspecific level is subtle, yet consideration of its pattern in the context of late Pleistocene land configuration in East Asia (Fig. 2) provides interesting taxonomic and biogeographical insights. In most species, for which samples are available from localities currently isolated by the sea but connected to each other during the late Pleistocene continental glaciation, the uncorrected p distances in pairs of such samples are so small (e.g. *T. toyamai*: 0.001 between Miyakojima [site #27 in Fig. 2] and Irabujima [#28]; *T. s. ocellatus*: 0.004 between Hong Kong [#11] and Lantau [#12]; *T. stejnegeri*: 0.004 between Taipei [#16], Fengyuan [#17] and Penghu [#18]). On the other hand, most pairs of conspecific samples from localities consistently isolated by the sea during the latest glaciation (Fig. 2) show greater distance values (i.e. 0.021–0.023 between the Takarajima [#13]–Kikajima [#14] and Kumejima [#15] samples of *T. smaragdinus*, and 0.034 between the Hapen [#3] and Lutao [#4] samples of *T. formosanus*: see also Fig. 3). These confirm the substantial influence of the late Pleistocene sea-level change on the formation of lizards' current geographical genetic pattern. Nevertheless, the pattern of intraspecific nucleotide replacements contradicts the putative late Pleistocene palaeogeography of *T. takydromoides*. In this species, a sample from Yakushima [#22] shows a much smaller p distance with samples from Tairajima [#19] and Nakanoshima [#20] (0.004–0.007) of the Tokara Islands than with samples of Kagoshima [#21] and other localities [#23–26] (0.035–0.040) to which Yakushima should have been connected during the latest glaciation (see also Fig. 3). This may reflect cryptic taxonomic diversity in this species. Further research, addressing this problem on the basis of information from nucleic DNA, is required.

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APPENDIX 1

Morphological characters used for the phylogenetic analyses by Arnold (1997) and distributions of their states among the *Takydromus* species. Character numbers and codes for their states are as follows:

1. Palatal processes of premaxilla slender and narrow and set at a fairly narrow angle to each other: no (0), or yes (1).
2. Premaxillary nasal process: narrow (0), or broad (1).
3. Nasal process of maxilla narrow and extended: no (0), or yes (1).
4. Tip of nasal process of maxilla embraced by frontal: no (0), or yes (1).
5. Head distinctly depressed: yes (0), or no (1).
6. Squamosal bone contacting parietal: no (0), or yes (1).
7. Posteroventral border of external exposure of coronoid bone forming an acute angle: no (0), or yes (1).
8. Posterior lateral teeth: third cusp often very small (0), or strongly tricuspid (1).
9. Usually 26 or more presacral vertebrae in males: yes (0), or no (1).
10. Snout elongate: no (0), or yes (1).
11. Postnasal scales: one (0), or sometimes two, the upper usually separating the supranasal scale from the first loreal (1).
12. First supraocular scale: usually present (0), or usually absent, with the second supraocular contacting the loreal (1).
13. Series of supraciliary granules usually present: yes (0), or no (1).
14. Number of supraciliary scales: usually more than three (0), or three (1).
15. Number of pairs of chin shields: four (0), or three (1).
16. Keeling on median gular scales extends anterior to line joining ears: no (0), or yes (1).
17. Dorsal scales on forebody: all small, not in obvious longitudinal rows (0), or more posterior scales large and in longitudinal rows but not those anterior to forelimbs (1), or dorsal scales large and in longitudinal rows extending beyond forelimbs on to neck (2).
18. Longitudinal rows if large dorsal scales between hind legs: eight to 10 (0), or six (1), or four (2).
19. Longitudinal series of dorsal scales bordering the mid-line of tail: not extending on to body (0), or extending on to body but diverging (1), or extending as far as nape without diverging (2).
20. Second longitudinal row of ventral scales on posterior belly usually broader than those bordering it: yes (0), or no (1).
21. Number of longitudinal ventral scale rows: eight (0), or six (1).
22. Enlarged lateral scales present: no (0), or yes (1).
23. Enlarged lateral scales adjoining ventral ones as long as these: no (0), or yes (1).
24. Ventral scales with longitudinal keels: none or outermost row of ventral scales only (0), or all ventral scales (1).
25. Series of smaller scales surrounding the preanal scale: continuous (0), or interrupted medially (1).
26. Preanal scale divided by a longitudinal suture: no (0), or yes (1).
27. Number of femoral pores on each side: three to five (0), or two (1), or one (2).
28. Number of rows of large scales running along underside of each thigh: three plus traces of fourth (0), or three well developed (1), or three with third reduced (2), or two (3).
29. Distal part of second row of large scales beneath thigh and main subtibial scale row with keels: no (0), or yes (1).
30. Internal tibial scale row usually with keels: no (0), or yes (1).
31. Pale dorsolateral stripes often present on body: no (0), or yes (1).
32. Dorsum distinctly green: no (0), or yes (1).
33. Outer lips of lobe sulci become basally directed flaps: no (0), or yes (1).
34. Hemipenis with sulci extending into lobes: no (0), or yes (1).
35. Transverse section of hemipenial lobe triskelial: no (0), or yes (1).

Appendix 1. Abbreviations for taxa are as follows. AC, hypothetical ancestor. KN, *Takydromus kuehnei*. IM, *T. intermedius*. DS, *T. dorsalis*. SV, *T. sylvaticus*. TY, *T. toyamai*. SD, *T. smaragdinus*. SA, *T. sauteri*. AM, *T. amurensis*. TM, *T. tachydromoides*. WT, *T. wolteri*. HS, *T. hsuelschaneensis*. FS, *T. formosanus*. SN, *T. stejnegeri*. ST, *T. septentrionalis*. KS, *T. khasiensis*. SL, *T. sexlineatus*.

Character	Sp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35			
AC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
KN	1	0	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	1	1	2	0	1	0	0	0	0,1	0	0	0	0	0	0	0	0	0	0	0	0	0
IM	-	0	0	-	-	1	1	0	1	0	1	0	0	0	0	0	0	2	1	2	0	1	0	0	-	0	0	0	1	2	0	0	0	0	0	0	0	0	0
DS	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	-	0	0	0	2	1	0	0	0	0	0	0	0	0	0
SV	1	0	0	1	0	-	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0,1	2	1	0	0	0	0	0	0	0	0	0
TY	1	0	0	0	-	0	0	1	1	1	1	1	0	0	0	1	1	2	1	0	0	1	0	1	0	1	0	1	2	1	1	1	0	0	1	0	0	0	
SD	1	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0,1	0	1	1	1	1	0	1	1	0	1	0,1	0,1	2	2,3	1	1	0	1	0	1	0	0	
SA	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0,1	0	0	1	1	0	0	1	1	0	1	0,1	0,1	2	3	1	1	0	1	0	1	0	0		
AM	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	2	1	2	0	0	0,1	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
TM	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	2	1	2	0	0	1	0	0	0,1	0	0	1	0	0,1	1	0	0	0	0	0	0	
WT	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0,1	0	0	2	0,1	2	1	0	1	0	0	0	0	0	2	0	0	0,1	1	0	0	0	0		
HS	0	1	0	0	-	0	0	1	1	0	0	0	0	0	0	1	0	2	1	2	1	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	
FS	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	2	1	2	1	0	1	1	1	1	0	0	1	1	1	1	0	0	0	0	0	0	
SN	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	2	1,2	2	1	0	1	1	1	1	0	0	2	1	1	1	1	1	0	0	0	0	
ST	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	1	2	2	2	1	0	1	0	1	0,1	0	0	2	1	1	1	1	0	0	0	0	0	
KS	0	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	2	2	2	1	0	1	1	1	1	0	0	1,2	1	1	1	1	0	0	0	0	0	
SL	0	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	2	2	2	1	0	1	1	1	1	1	0	1,2	1	1	1	1	0	0	0	0	0	

*Character states newly recovered in the present study.

APPENDIX 2

SPECIMENS EXAMINED

Numerals in parentheses following locality names of ingroup samples correspond to those in Figure 2.

Lacerta vivipara. Russia: Sakhalin: KUZ R18091.

Podarcis muralis. Locality unknown (purchased from pet dealer): KUZ R34477.

Takydromus amurensis. Korea: Odaesan [1]: KUZ R46684, 46715.

T. dorsalis. Japan: Okinawa Pref. Iriomotejima Is. [2]: KUZ R33089, 48630.

T. formosanus. Taiwan: Taipei Co. Hapen [3]: KUZ R19204, 19236; Taitung Co. Lutao Is. (4): KUZ R32572, 32573.

T. hsuehshanensis. Taiwan: Nantou Co. Hehuanshan [5]: KUZ R19238, 19241.

T. kuehnei. Taiwan: Nantou Co. Lushan [6]: KUZ R34760.

T. sauteri. Taiwan: Taitung Co. Lanyu Is. [7]: KUZ R32569, 32571.

T. septentrionalis. Taiwan: Matsu Island Group: Nankan Is. [8]: KUZ R33790, 33791; locality unknown (purchased from pet dealer): KUZ R20381, 20382.

T. sexlineatus sexlineatus. Malaysia: Sarawak State (Borneo): Kuchin [9]: KUZ R12412, 12517.

T. sexlineatus ocellatus. Thailand: Rayong Prov., Khao Wong [10]: KUZ R32834, 32836; China: Hong Kong: Hong Kong Is. [11]: KUZ R47218; Lantau Is. [12]: KUZ R33814.

T. smaragdinus. Japan: Kagoshima Pref. Takarajima Is. [13]: KUZ R8285; Kikaijima Is. [14]: KUZ R18855; Okinawa Pref. Kumejima Is. [15]: KUZ R20902.

T. stejnegeri. Taiwan: Taipei Co. Taipei City [16]: KUZ R19198; Taichung Co. Fengyuan [17]: KUZ R19202; Penghu Co. Penghu Is. [18]: KUZ R19321, 19331.

T. tachydromoides. Japan: Kagoshima Pref. Tairajima Is. [19]: KUZ R8287; Nakanoshima Is. [20]: KUZ R19188, 19191; Kagoshima City [21]: KUZ R34523; Yakushima Is. [22]: KUZ R34533; Kyoto Pref. Kyoto City [23]: KUZ R19218; Nagasaki Pref. Iki Is. [24]: KUZ R33907; Ibaraki Pref. Tokai [25]: KUZ R50453; Ishikawa Pref. Shiga-cho [26]: KUZ R46908.

T. toyamai. Japan: Okinawa Pref. Miyakojima Is. [27]: KUZ R18842; Irabujima Is. [28]: KUZ R21172.

T. wolteri. Korea: Cheju Is. [29]: KUZ R52293.

