

# Estimation of the Origin of *Polypedates leucomystax* (Amphibia: Anura: Rhacophoridae) Introduced to the Ryukyu Archipelago, Japan<sup>1</sup>

Noribiro Kuraishi,<sup>2</sup> Masafumi Matsui,<sup>2,3</sup> and Hidetoshi Ota<sup>4</sup>

**Abstract:** We attempted to estimate the origin of the exotic frog *Polypedates leucomystax* in the Ryukyu Archipelago. This species was first found in 1964 just in front of the U.S. military base at Kadena on Okinawajima Island and currently has established feral populations on more than 20 islands. We conducted phylogenetic analyses using mitochondrial DNA sequences of the cytochrome *b* gene. Samples of *P. leucomystax* from five islands of the Ryukyus had a single haplotype, which was identical to that of a Philippine sample but quite different from haplotypes of Vietnamese samples. Samples of *P. megacephalus* from Taiwan formed a clade different from the *P. leucomystax* clade. From these results, *P. leucomystax* in the Ryukyus seems to have originated through accidental transportation of very few individuals with military cargo from somewhere around the Philippines.

IT IS WIDELY ACCEPTED that introduced species often have adverse effects on native ecosystems (Williamson 1996), and small, closed ecosystems like those on islands are especially prone to be affected by such invaders (e.g., Diamond and Case 1986, Case et al. 1992, Cowie 1992). The Ryukyu Archipelago of southern Japan, located in the humid subtropical region of East Asia, is famous for its high herpetological endemism (Ota 1998, 2000), and discoveries of endemic taxa are still continuing (e.g., Matsui et al. 2005, 2007, Toda et al. 2008). However, three exotic frogs, *Bufo marinus* Linnaeus, 1758 (= *Rhinella marina* [Linnaeus, 1758]), *Rana*

*catesbeiana* Shaw, 1802 (= *Lithobates catesbeianus* [Shaw, 1802]), and *Polypedates leucomystax* (Gravenhorst, 1829), have been recorded from several islands of the archipelago (Maeda and Matsui 1999, Ota 1999, Ota et al. 2004). Although these species were designated as Invasive Alien Species in 2006 by the Ministry of Environment (Japan), ecological surveys for these species have just been started. Therefore, information regarding their ecology is meager, and their effect on native fauna is poorly understood.

Of these exotic frogs introduced into the Ryukyu Archipelago, *P. leucomystax* is originally a tropical species ranging widely over most of Southeast Asia (Maeda and Matsui 1999). The species was first found in 1964 near the U.S. military base at Kadena, which is located in the south-central part of Okinawajima Island, the central Ryukyus (Kura-moto 1965). Since then, the species has been recorded from various localities within Okinawajima Island (e.g., Utsunomiya 1977, Chigira 1984, Moriguchi 1988), as well as from several adjacent islands (Toyama 1981). In reviewing distributions of amphibians and reptiles in the Ryukyu Archipelago, Maenono and Toda (2007) recorded *P. leucomystax* from no less than 21 islands of over 40 of the Okinawa Group and Miyako Group. The range of the species is still expanding within the Ryukyus, reaching Ishigakijima Island of the Yaeyama Group and Kita-Daitojima Is-

<sup>1</sup> Fieldwork in this study was partially supported by a grant-in-aid from the Research Institute for Humanity and Nature (Project Leader: Tokushiro Takaso), and laboratory work was partially supported by a grant under The Monbusho/JSPS Scientific Research Program No. 15370038 to M.M. Manuscript accepted 21 October 2008.

<sup>2</sup> Graduate School of Human and Environmental Studies, Kyoto University, Sakyo, Kyoto 606-8501, Japan.

<sup>3</sup> Corresponding author (e-mail: fumi@zoo.zool.kyoto-u.ac.jp).

<sup>4</sup> Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.

land of the Daito Group (Ota et al. 2008). Because the species is often closely associated with human habitation (e.g., Inger 1954, Maeda and Matsui 1989, Inger and Stuebing 2005), it could easily be transported from island to island via transport vessels (Brown and Alcalá 1970).

Although the influence of *P. leucomystax* on the native ecosystem of the Ryukyu Archipelago remains unknown, those impacts would likely be negative, at least on native frog species with similar life histories, such as *Rhacophorus viridis* (Hallowell, 1861) and *R. owstoni* (Stejneger, 1907). It is therefore desirable to eradicate this invasive species from islands of the Ryukyus. To achieve this, we require information on various aspects of the frog and its invasion history. For this purpose, genetic analyses, especially of rapidly evolving genes in the mitochondrial DNA

(mtDNA), are considered suitable. Among these genes, the cytochrome *b* (*cyt-b*) gene has been used successfully to clarify relationships among conspecific populations in some other frog species from the Ryukyus (Matsui et al. 2005, in press). In this study, therefore, we used *cyt-b* to estimate the origin of the current feral populations of *P. leucomystax* in the Ryukyu Archipelago.

#### MATERIALS AND METHODS

We used 14 tissue samples of *P. leucomystax* from five islands in the Ryukyu Archipelago (Figure 1, Table 1). To specify affinity of the current Ryukyu populations, we selected samples from Vietnam (three tissue samples from three localities) and the Philippines (one tissue sample) for comparisons. This is because U.S. military bases existed in those

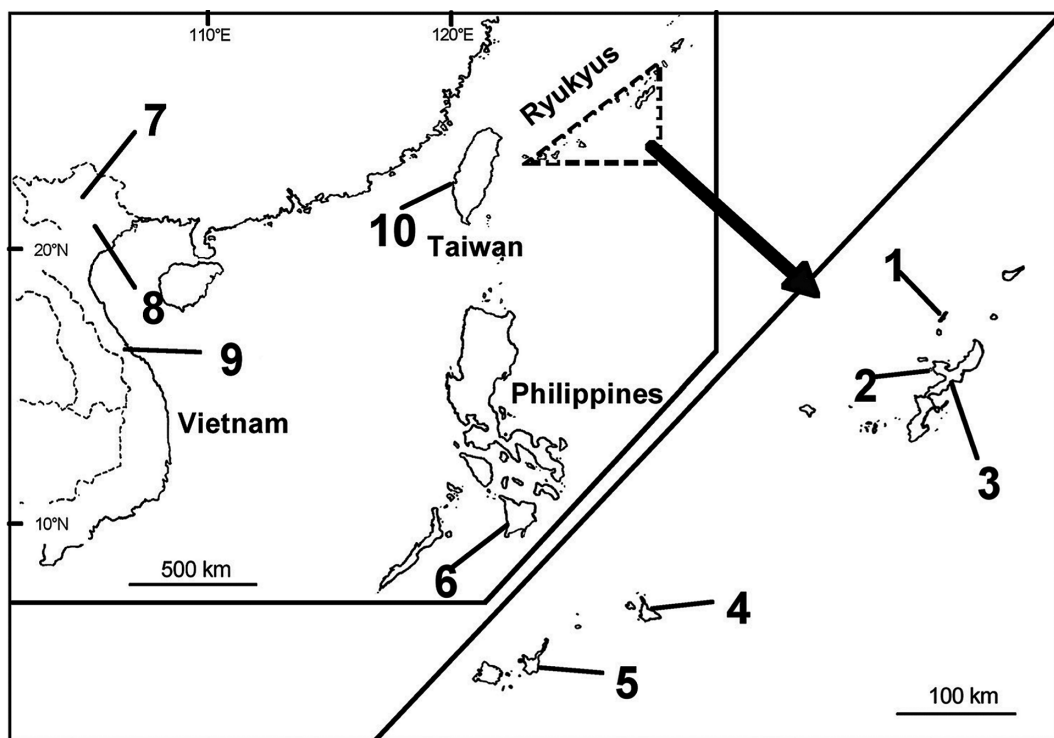


FIGURE 1. Map of Southeast Asia and East Asia including the Ryukyu Archipelago (inset), showing sampling localities of *Polypedates leucomystax* and *P. megacephalus* used in this study. 1, Iheyajima; 2, Sesokojima; 3, Okinawajima; 4, Miyakojima; 5, Ishigakijima; 6, Panay Island; 7, Tam Dao; 8, Hanoi; 9, Hue; 10, Taiwan.

TABLE 1  
Samples Used in This Study and GenBank Accession Numbers

Species	Locality <sup>a</sup>	Voucher <sup>b</sup>	Accession No.
<i>Polypedates leucomystax</i>	Ryukyu, Iheyajima (1)	KUHE 32283	AB451701
	Ryukyu, Iheyajima (1)	KUHE 32284	AB451702
	Ryukyu, Sesokojima (2)	KUHE 29803	AB451703
	Ryukyu, Okinawajima (3)	KUHE 41185	AB451704
	Ryukyu, Okinawajima (3)	KUHE 34657	AB451705
	Ryukyu, Okinawajima (3)	KUHE 22556	AB451706
	Ryukyu, Okinawajima (3)	KUHE 35355	AB451707
	Ryukyu, Okinawajima (3)	KUHE 9576	AB451708
	Ryukyu, Okinawajima (3)	KUHE 10087	AB451709
	Ryukyu, Miyakojima (4)	KUHE 37792	AB451710
	Ryukyu, Miyakojima (4)	KUHE 37793	AB451711
	Ryukyu, Miyakojima (4)	KUHE 37794	AB451712
	Ryukyu, Ishigakijima (5)	KUZ R62733	AB451713
	Ryukyu, Ishigakijima (5)	KUZ R62683	AB451714
	Philippines, Panay Island (6)	KUHE 34396	AB451715
	Vietnam, Tam Dao (7)	KUHE 38476	AB451716
	Vietnam, Hanoi (8)	KUHE 38481	AB451717
	Vietnam, Hue (9)	KUHE 40544	AB451718
	Taiwan (10)	KUHE 32842	AB451719
	Taiwan (10)	KUZ 31071	AB451720
<i>Polypedates megacephalus</i>	Taiwan (10)	KUZ 31072	AB451721
	Taiwan (10)	KUHE 12971	AB451722
<i>Rhacophorus schlegelii</i>	Japan, Hiroshima	GenBank sequence	AB202078
<i>Buergeria buergeri</i>	Japan, Hiroshima	GenBank sequence	AB127977

<sup>a</sup> Locality numbers correspond to those in Figure 1.

<sup>b</sup> KUHE, Graduate School of Human and Environmental Studies, Kyoto University; KUZ, Department of Zoology, Graduate School of Science, Kyoto University.

countries in the 1960s. We also used four tissue samples of *P. megacephalus* Hallowell, 1861, from Taiwan because this species closely resembles *P. leucomystax* morphologically (Matsui et al. 1986), and Taiwan is geographically closest to the Ryukyu Archipelago. For the outgroup, we used GenBank data of two rhacophorids, *Buergeria buergeri* (Temminck & Schlegel, 1838) (AB127977) and *Rhacophorus schlegelii* (Günther, 1858) (AB202078) (Table 1).

We extracted total DNA from a small amount of tissue from each ethanol-preserved or frozen specimen using standard phenol-chloroform extraction procedures (Hillis et al. 1996). Amplifications were done by polymerase chain reaction (PCR) with two primers (made in this study) of tGluL: 5'-TTC TTA CAA GGA TTT TAA CCT AGAC-3' and HCytbH2: 5'-GCC AAT TAT AAT AAA TGG GTC TTC TAC

TG-3' to obtain 1,074 bp of cyt-*b* and tRNA<sup>Glu</sup> gene. We sequenced the amplified fragments by an automated DNA sequencer (ABI PRISM 3130) using PCR primers and two additional primers (Rrhaco: 5'-ATG TAG AAT AGG SGT GRA ATG GMA YTT TGT-3' and HCytbL1: 5'-CTA TTT CTT CAC CAA ACA GGC TCA TC-3'; made in this study). Newly obtained sequences were deposited in GenBank (Table 1).

We obtained sequence data for each sample and manually adjusted them by eye using Chromas Pro (Technelysium Pty Ltd., Tewantin, Australia) software. We also performed alignments of all data by Clustal W in the BioEdit software (Hall 1999). We then obtained sequences of about 1,074 bp (cyt-*b*: 1,027 bp; tRNA<sup>Glu</sup>: ca. 47 bp), of which we used only the stable 1,027 bp of cyt-*b* for phylogenetic analyses.

We used three methods for estimating

phylogenetic relationships: (1) maximum parsimony (MP) using a heuristic search with the tree bisection reconnection (TBR) branch-swapping algorithm, 100 random addition replicates, and an equal weighting option for transitions and transversions; (2) maximum likelihood (ML) analysis based on the substitution model and phylogenetic parameters, chosen by the program Modeltest 3.06 (Posada and Crandall 1998) as the best option in a hierarchical likelihood ratio test (hLRT), and using a heuristic search with the TBR branch-swapping algorithm and 10 random additional replicates; and (3) Bayesian inference (Huelsenbeck et al. 2001, Rannala and Yang 1996) with the model derived from an hLRT in MrModeltest v2.2 (Nylander 2004), using four simultaneous Metropolis-coupled Monte Carlo Markov chains for 1,000,000 generations and sampling a tree every 100 generations. To determine stationary trees, we discarded the initial 1,000 trees for burn-in and used remaining trees to estimate Bayesian posterior probabilities (BPP).

MP and ML analyses were conducted using PAUP\*4.0b (Swofford 2002), and pairwise comparisons of uncorrected sequence divergences (p-distance) were also calculated using PAUP. Bayesian analysis was conducted using MrBayes (Huelsenbeck and Ronquist 2001). The robustness of the MP and ML trees was tested using bootstrap analyses (Felsenstein 1985), with 1,000 (MP) and 100 (ML) replicates (Hedges 1992). We regarded tree topologies with bootstrap values greater than 70% as sufficiently supported (Hillis and Bull 1993). For the Bayesian analysis, we considered BPP greater than 95% as significant support (Huelsenbeck et al. 2001, Leaché and Reeder 2002, Huelsenbeck and Rannala 2004).

#### RESULTS

We obtained 24 sequences of 1,027 bp for *cyt-b*, of which 412 bp were variable and 265 bp were parsimoniously informative. The best substitution model derived from Modeltest was HKY+G (Hasegawa et al. 1985). MrModeltest also concluded that HKY+G

fit the data, so we applied this model to each analysis.

We obtained four most parsimonious trees with 635 evolutionary steps, with a consistency index (CI) of 0.828 and a retention index (RI) of 0.888. The likelihood values of the ML tree and the consensus tree in the Bayesian analysis were  $\ln L = -3913.36$  and  $\ln L = -3909.94$ , respectively. The burn-in in the Bayesian analysis occurred before 10,000 generations (data not shown); we discarded the first 100,000 generations. The three phylogenetic analyses yielded slightly different but essentially the same topologies, thus the consensus MP tree is shown in Figure 2. The following relationships were indicated by all three analyses as statistically reliable: (1) monophyly of *Polypedates* with respect to the outgroup *Buergeria* and *Rhacophorus* (100/100/100%: MP bootstrap value/ML bootstrap value/BPP); (2) dichotomy of *Polypedates* into *P. leucomystax* from the Ryukyu, Vietnam, and the Philippines (99/100/99%) and *P. megacephalus* from Taiwan (100% in all); and (3) monophyly of the Ryukyu and Philippine samples against the Vietnamese samples within *P. leucomystax* (100% in all). The Vietnamese clade was supported only by MP (79%).

A single haplotype was shared by all Ryukyu samples and also by the Philippine sample. In contrast, this haplotype was different from the Vietnamese haplotypes. The average p-distance between the Ryukyu samples and Taiwanese samples of *P. megacephalus* was 19.2%, and that between the Ryukyu and Vietnamese samples was 10.1%.

#### DISCUSSION

Previous authors (e.g., Toyama 1981, Maeda and Matsui 1989) considered the original localities of the Ryukyu populations of *P. leucomystax* to be somewhere in Indochina. This assertion was based on the volume of traffic between the military base on Okinawajima Island and those in Vietnam and adjacent regions of Indochina. In the helminthological survey of specimens of *P. leucomystax* from Okinawajima Island, Hasegawa (1993) re-

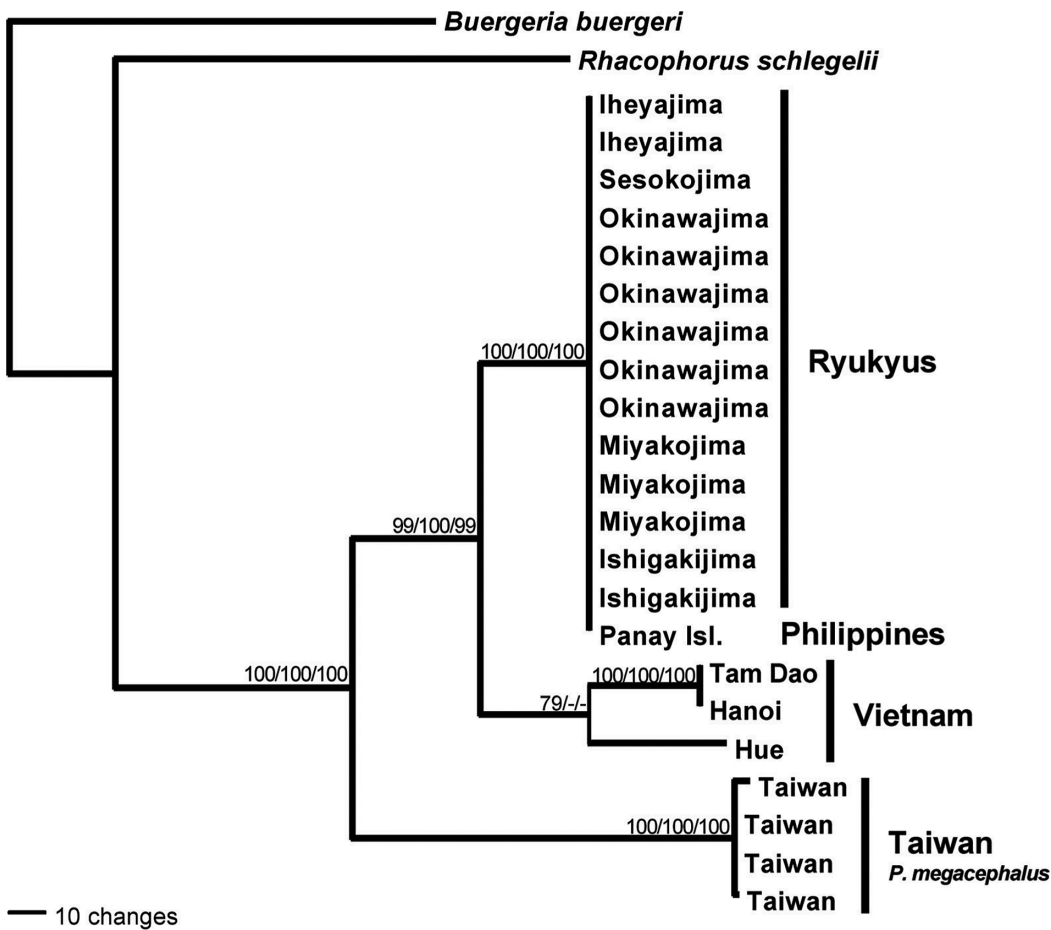


FIGURE 2. The consensus maximum parsimony tree of 1,027 bp of the cytochrome *b* gene for all samples of *Polypedates leucomystax*, *P. megacephalus*, and outgroups. Numerals above each branch represent bootstrap supports (%) in MP (1,000 times) and ML (100), and Bayesian inference.

ported a nematode species, *Raillietnema rhacophori* Yuen, 1965, that was otherwise known only from Malaysia. Following Maeda and Matsui (1989), Hasegawa (1993) suspected that *R. rhacophori* occurs in Indochina. However, the results of our study do not support the Indochinese origin of the current Ryukyu populations of *P. leucomystax*. In spite of the relatively high evolutionary rate of the *cyt-b* gene, which usually contains some sequence variations even within a single species (Austin et al. 2004), all samples from the Ryukyus possessed an identical haplotype, which was

shared exclusively with the Philippine sample. By contrast the three Vietnamese (Indochinese) samples examined showed a substantial divergence, forming a single clade only in the MP tree, where they were sister to the Ryukyu-Philippine clade. Also in spite of being geographically close to each other, Taiwan samples showed a substantial divergence from samples from the Ryukyus.

Acoustic evidence also favors the association of the Ryukyu populations with the Philippine, rather than Vietnamese, populations. According to Maeda and Matsui (1999), the

mating call of the population of *P. leucomystax* from Okinawajima Island has a note lasting about 0.15 sec. This note duration is closer to that of the Philippine populations (0.15–0.24 sec [Brozoska et al. 1986]) than to that of the Vietnamese populations (0.33 sec or 0.64 sec [Trépanier et al. 1999]), although our DNA samples were obtained from localities not necessarily identical to those where those calls were recorded. We nevertheless conclude that the Ryukyu populations of *P. leucomystax* most likely derived from a recent arrival from the Philippines rather than Indochina.

In their review of nonindigenous amphibians hitherto found from Guam, Christy et al. (2007a,b) assumed several pathways for their invasion, such as intentional introduction as a pest control agent, accidental stowing away via maritime or air-transport vessels, and unintentional transportation through horticultural, agricultural, and aquacultural trading activities. With respect to *P. leucomystax* in the Ryukyus, the current populations are considered, as already mentioned, to have derived from individuals accidentally introduced to Okinawajima Island by military vessels (Toyama 1981, Maeda and Matsui 1989, Ota 1999). The initial capture of this species from the archipelago was adjacent to the U.S. Air Force base at Kadena in south-central Okinawajima Island (Kuramoto 1965), a place relatively distant from nonmilitary ports and airports. The idea of accidental transportation via military vessels is not new and is circumstantially supported by a recent accidental transportation of *P. leucomystax* from Thailand to Guam by a military cargo aircraft (Wiles 2000). This recent introduction indicates that the species can survive as a stowaway. In addition, the Kadena Air Base has had much contact with military aircraft from military bases in Southeast Asia, including the Philippines in the 1960s (Kadena Town Board of Education 1983), making it possible for this pathway to exist.

No genetic variation was detected among samples from five islands of the Ryukyu Archipelago. This suggests that the founding individuals were genetically uniform, possibly limited in number, and from a single locality

or at most a few closely located localities. Range extension within Okinawajima Island would not have been difficult, but due to the relative intolerance of amphibians to seawater, oceanic dispersals to other islands must also have occurred in association with human activities. Indeed, this species is known to be a human commensal (Maeda and Matsui 1989, Inger and Stuebing 2005) and thus could be opportunistically transported in cargo on ships and planes from island to island. In spite of closer geographic locations to Okinawajima Island of some Kagoshima Prefecture islands (Yoronjima Island, Okinoerabujima Island, etc.), the strict limitation of the range of *P. leucomystax* to islands of Okinawa Prefecture supports the assumption of human-mediated dispersals of this frog within the Ryukyus, as in the case of some Caribbean *Eleutherodactylus* species (e.g., Kaiser 1992).

The chronology of *P. leucomystax* in the Ryukyu Archipelago likely started in the 1960s. In the mid-1960s, a few individuals were probably accidentally introduced to the U.S. base at Kadena from a single or a few close localities in the Philippines (but see the next paragraph), most probably by military transportation. The species established and rapidly extended its range, first within Okinawajima Island and a few adjacent islands, and then to the more distant islands of Okinawa Prefecture, again most likely as stowaways (see Ota [1999] and Ota et al. [2004] for detailed chronologically arranged geographic records of this species within the Ryukyus).

This study only included one individual of *P. leucomystax* from the Philippines. That country, however, contains many islands, on most of which the species occurs (Inger 1954). Therefore, we cannot specify the origin of the Ryukyu populations within the Philippines. *Polypedates leucomystax* is also distributed on several large islands near the Philippines, such as Borneo, Sulawesi, and Java (type locality), and some Philippine populations may have derived from outside the country (Brown and Alcalá 1970). To identify the specific location of origin, further studies on genetic variations are needed, not only for additional Philippine populations but also

for Indonesian and Malaysian populations. Whether or not the parasitic nematode *Railletnema rhacophori* found in Okinawajima and Malaysia (Hasegawa [1993], as mentioned earlier) also occurs in the Philippine populations of *P. leucomystax* requires investigation. From the viewpoint of conservation of native biodiversity in the Ryukyus, serious considerations and efforts should be made to prevent further range extension of this frog through human activities (Kraus and Campbell 2002).

#### ACKNOWLEDGMENTS

We are grateful to T. Hikida, S. Ikeda, I. Katsube, S. Katsuren, H. Nagaoka, H. Nakaza, T. Shimada, T. Sugahara, I. Takiguchi, H. T. Thong, M. Tsunoda, and N. Yoshikawa for offering us valuable samples and/or helping us in the field survey. We also thank A. Tominaga for laboratory assistance.

#### Literature Cited

- Austin, J. D., S. C. Loughheed, and P. T. Boag. 2004. Discordant temporal and geographic patterns in maternal lineages of eastern north American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). *Mol. Phylogenet. Evol.* 32:799–816.
- Brown, W. C., and A. C. Alcala. 1970. The zoogeography of the herpetofauna of the Philippine Islands, a fringing archipelago. *Proc. Calif. Acad. Sci.* 38:105–130.
- Brozoska, J., G. Joermann, and A. C. Alcala. 1986. Structure and variability of the calls of *Polypedates leucomystax* (Amphibia: Rhacophoridae) from Negros, Philippines. *Siliman J.* 33:87–103.
- Case, T. J., D. T. Bolger, and A. D. Richman. 1992. Reptilian extinctions: The last ten thousand years. Pages 91–125 in P. L. Fiedler and S. K. Jain, eds. *Conservation biology*. Chapman and Hall, New York.
- Chigira, Y. 1984. Records of *Polypedates leucomystax* at the northern part of Okinawajima. *Akamata* 2:2 [in Japanese].
- Christy, M. T., C. C. Clark, D. E. Gee II, D. Vice, D. S. Vice, M. P. Warner, C. L. Tyrrell, G. H. Rodda, and J. A. Savidge. 2007a. Recent records of alien anurans on the Pacific island of Guam. *Pac. Sci.* 61:469–483.
- Christy, M. T., J. A. Savidge, and G. H. Rodda. 2007b. Multiple pathways for invasion of anurans on a Pacific island. *Divers. Distrib.* 13:598–607.
- Cowie, R. H., ed. 1992. The impact of alien species on island ecosystems: Extended abstracts of a symposium, 30 May 1991, Honolulu, Hawai'i, XVII Pacific Science Congress. *Pac. Sci.* 46:383–404.
- Diamond, J. M., and T. J. Case. 1986. Overview: Introduction, extinctions, exterminations, and invasions. Pages 65–79 in J. M. Diamond and T. J. Case, eds. *Community ecology*. Harper and Row, New York.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.
- Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41:95–98.
- Hasegawa, H. 1993. *Railletnema rhacophori* Yuen, 1965 (Nematoda: Cosmocercidae) collected from a frog, *Polypedates leucomystax*, on Okinawajima, Japan. *Biol. Mag. Okinawa* 31:15–19.
- Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22:160–174.
- Hedges, S. B. 1992. The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. *Mol. Biol. Evol.* 9:366–369.
- Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for confidence in phylogenetic analyses. *Syst. Biol.* 42:182–192.
- Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. Nucleic acids IV: Sequencing and cloning. Pages 321–378 in D. M. Hillis, C. Moritz, and B. K. Mable, eds. *Molecular systematics*. Sinauer Associates, Sunderland, Massachusetts.
- Huelsenbeck, P., and B. Rannala. 2004. Frequentist properties of Bayesian posterior

- probabilities of phylogenetic trees under simple and complex substitution models. *Syst. Biol.* 53:904–913.
- Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- Huelsenbeck, J. P., F. Ronquist, R. Nielsen, and J. P. Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294:2310–2314.
- Inger, R. F. 1954. Systematics and zoogeography of Philippine Amphibia. *Fieldiana Zool.* 33:181–531.
- Inger, R. F., and R. B. Stuebing. 2005. A field guide to the frogs of Borneo. 2nd ed. Natural History Publications, Kota Kinabalu, Malaysia.
- Kadena Town Board of Education, ed. 1983. History of Kadena Town. Kadena Town Office, Okinawa [in Japanese].
- Kaiser, H. 1992. The trade-mediated introduction of *Eleutherodactylus martinicensis* (Anura: Leptodactylidae) on St. Barthèlèmy, French Antilles, and its implications for Lesser Antillean biogeography. *J. Herpetol.* 26:264–273.
- Kraus, F., and E. W. Campbell. 2002. Human-mediated escalation of a formerly eradicable problem: The invasion of Caribbean frogs in the Hawaiian Islands. *Biol. Invasions* 4:327–332.
- Kuramoto, M. 1965. A record of *Rhacophorus leucomystax* from the Ryukyu Islands. *Bull. Fukuoka Gakugei Univ. Part III* 15:59–61.
- Leaché, A. D., and T. W. Reeder. 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): A comparison of parsimony, likelihood, and Bayesian approaches. *Syst. Biol.* 51:44–68.
- Maeda, N., and M. Matsui. 1989. Frogs and toads of Japan. Bun-ichi Sogo Shuppan, Tokyo [in Japanese with English abstract].
- . 1999. Frogs and toads of Japan. Rev. ed. Bun-ichi Sogo Shuppan, Tokyo [in Japanese with English abstract].
- Maenosono, T., and M. Toda. 2007. Distributions of amphibians and terrestrial reptiles in the Ryukyu Archipelago: A review of published records. *Akamata* 18:28–46 [in Japanese].
- Matsui, M., H. Ito, T. Shimada, H. Ota, S. K. Saidapur, W. Khonsue, T. Tanaka-Ueno, and G. F. Wu. 2005. Taxonomic relationships within the Pan-Oriental narrow-mouth toad *Microhyla ornata* as revealed by mtDNA analysis (Amphibia, Anura, Microhylidae). *Zool. Sci. (Tokyo)* 22:489–495.
- Matsui, M., T. Seto, and T. Utsunomiya. 1986. Acoustic and karyotypic evidence for specific separation of *Polypedates megacephalus* from *P. leucomystax*. *J. Herpetol.* 20:483–489.
- Matsui, M., T. Tanaka-Ueno, and H. Ota. A molecular approach to the speciation of amphibians in the Ryukyu Archipelago. *Biol. J. Linn. Soc.* (in press).
- Matsui, M., M. Toda, and H. Ota. 2007. A new species of frog allied to *Fejervarya limnocharis* from the southern Ryukyus, Japan (Amphibia: Ranidae). *Curr. Herpetol.* 26:65–79.
- Moriguchi, H. 1988. A record of *Polypedates leucomystax* from the southern part of Okinawajima, Ryukyu Archipelago. *Akamata* 5:1 [in Japanese].
- Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.
- Ota, H. 1998. Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications. *Res. Popul. Ecol. (Kyoto)* 40:189–204.
- . 1999. Introduced amphibians and reptiles of the Ryukyu Archipelago, Japan. Pages 432–452 in G. H. Rodda, Y. Sawai, D. Chiszar, and H. Tanaka, eds. Problem snake management: The habu and the brown treesnake. Cornell University Press, Ithaca, New York.
- . 2000. The current geographic faunal pattern of reptiles and amphibians of the Ryukyu Archipelago and adjacent regions. *Tropics* 10:51–62.
- Ota, H., M. Toda, G. Masunaga, A. Kikukawa, and M. Toda. 2004. Feral populations of amphibians and reptiles in the Ryukyu Archipelago, Japan. *Global Environ. Res.* 8:133–143.



- Ota, H., M. Tsunoda, H. Nakaza, and A. Nakayama. 2008. Records of the exotic frog, *Polypedates leucomystax*, from Ishigakijima Island of the Yaeyama Group and Kitadaitojima Island of the Daito Group, Ryukyu Archipelago, Japan. *Akamata* 19:44–48 [in Japanese].
- Posada, D., and K. A. Crandall. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- Rannala, B., and Z. Yang. 1996. Probability distribution of molecular evolution trees: A new method of phylogenetic inference. *J. Mol. Evol.* 43:304–311.
- Swofford, D. L. 2002. PAUP\*: Phylogenetic analysis using parsimony (\*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Toda, M., S. Sengoku, T. Hikida, and H. Ota. 2008. Description of two new species of the genus *Gekko* (Squamata: Gekkoni-  
dae) from the Tokara and Amami Island Groups in the Ryukyu Archipelago, Japan. *Copeia* 2008:452–466.
- Toyama, M. 1981. Preliminary reports on the herpetological fauna of the Okinawa Islands, Ryukyu Archipelago (I). *Bull. Okinawa Pref. Mus.* 7:1–8 [in Japanese with English summary].
- Trépanier, T. L., A. Lathrop, and R. W. Murphy. 1999. *Rhacophorus leucomystax* in Vietnam with acoustic analyses of courtship and territorial calls. *Asiatic Herpetol. Res.* 8:102–106.
- Utsunomiya, T. 1977. On *Rhacophorus leucomystax* in Genga, Okinawa. *Jpn. J. Herpetol.* 7:45 [in Japanese].
- Wiles, G. J. 2000. Recent records of reptiles and amphibians accidentally transported to Guam, Mariana Islands. *Micronesica* 32:285–287.
- Williamson, M. 1996. Biological invasions. Chapman and Hall, London.

