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Taxonomic Status of the Legless Lizard *Ophisaurus* (Squamata: Anguidae) in Taiwan: Molecular Data, Morphology, and Literature Review

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Si-Min Lin, Wen-Shiang Chang, Szu-Lung Chen, Gaus Shang and Kuang-Yang Lue (2003) Taxonomic status of the legless lizard *Ophisaurus* (Squamata: Anguidae) in Taiwan: molecular data, morphology, and literature review. *Zoological Studies* **42**(3): 411-419. The legless lizard genus *Ophisaurus* is in the family Anguidae. Two species are currently recognized in Taiwan: *O. harti* with blue dorsal markings, and *O. formosensis* with uniform dorsal coloration. However, due to the secretive life history and extremely few records, the taxonomic status of *O. formosensis* remains problematic. In order to resolve this controversy, molecular and morphology data were obtained from 14 *Ophisaurus* individuals with blue dorsal markings (*O. harti*) and 5 without markings (*O. formosensis*). Complete *12S rRNA* and partial *cytochrome b* sequences revealed no genetic differentiation between these 2 color morphs; in addition, some of their haplotypes are shared. We reviewed the related literature and evaluated 8 different morphological characters that were once mentioned or still apply for species recognition, but none of them provided a diagnostic distinction between these 2 color morphs in our samples. Furthermore, some characters of *O. formosensis* proposed by recent herpetologists are incongruent with the description of the holotype in the original paper. Based on their sympatric distribution, we conclude that there is only 1 *Ophisaurus* species in Taiwan, and *O. formosensis* should be a synonym of *O. harti*.
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Key words: Legless lizards, *Ophisaurus*, Anguidae, Taiwan.

The legless glass lizard genus, *Ophisaurus* Daudin, 1803 in the family Anguidae, is distributed throughout Nearctic and Old World regions, including eastern North America, eastern and western Asia, and southern Europe (Macey et al. 1999). They are characterized by their legless, elongated, and anguimorph body shape. Two *Ophisaurus* species are currently recognized in Taiwan, inhabiting dense and slightly disturbed natural forests from 500 to 2000 m in elevation (Lin and Cheng 1990, Lue and Lai 1991, Lue et al. 1999). *Ophisaurus harti* Boulenger, 1899, described from Fujian, southeastern China, and distributed also in Vietnam and Taiwan, is characterized by its dorsal blue markings (Fig. 1A). Although records of this species are not common, it is widely distributed throughout Taiwan where natural vegetation remains. *Ophisaurus formosensis*, described by Kishida (1930) as an endemic species in Taiwan, is distinguished from *O. harti* by its uniform dorsal coloration with no blue markings (Fig. 1B). It is probably sympatrically distributed with *O. harti*, but there are extremely few records (Lue et al. 1987, Lue 1990, Lue et al. 1999).

According to Kishida's original description in 1930, coloration was the major difference used to distinguish *O. formosensis* and *O. harti*. Another 2 characters in his statements included a lack of black dots on the head and the shape of the occipital plate (Table 1). However, some other morpho-

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logical differences were mentioned by later herpetologists, including the number of dorsal scale rows and other lepidosis characters on the head (Wang and Wang 1956, Liu-Yu 1970, Wang and Liang 1976, Zhao et al. 1999). At least 5 other lepidosis characters were proposed (Table 1), and contradictions among some of these statements led to uncertainty in defining these 2 species. Due to the secretive life history and the limited number of specimens, the eastern Asian *Ophisaurus* is poorly studied. In the case of *O. formosensis*, observations are so rare that one would rarely expect to collect any individuals intentionally. In the latest review of East Asian *Ophisaurus*, Brygoo (1987) briefly referred to Wang and Wang's description (1956) and left the status of this species as uncertain. Therefore, the validity of *O. formosensis* remains doubtful (Lin and Cheng 1990).

In the last several years, several fresh roadkill specimens identified as *O. formosensis* were collected, offering a valuable opportunity for a revision of this rare species. Individuals with blue markings were compared to those without markings using morphological characters, mitochondrial DNA sequences, and a literature review, in order to reevaluate the taxonomic status of *Ophisaurus* in Taiwan.

MATERIALS AND METHODS

Sample collection, PCR, and DNA sequencing

From 1997 to 2001, *Ophisaurus* specimens were obtained from 9 localities in Taiwan (Table 2; Fig. 2). Most specimens were obtained as road kills near their natural habitats. Species identification followed Kishida (1930): *O. harti* with blue dorsal markings, and *O. formosensis* with uniform dorsal coloration and no blue markings. In total, 5 *O. formosensis* and 14 *O. harti* individuals were included for mitochondrial DNA sequencing. Two other anguid species including 1 *O. gracilis* from Yunnan Province, China, and 2 *Pseudopus apodus* from southern Europe served as outgroups. These specimens are now preserved at the Department of Biology, National Taiwan Normal University.

Genomic DNA was extracted from muscle tissue by a standard phenol/chloroform protocol. The complete mitochondrial *12S ribosomal RNA* (rRNA) gene and the flanking valine *tRNA* gene were amplified as a single fragment with primers designed by Wang et al. (2000): PL1: 5'-AGTCTGCTCAAAAAGATTAATGTTAA-3', and PH1: 5'-TCTTGGTCTGAAACCTCAGTTACCTA-3'. The partial *cytochrome b* gene of about 750 bp in length was amplified with primers designed from the consensus sequence of several Squamata species: PL2: 5'-CCMTCMAACMTYTCMDYWTKRTGAAA-3', and PH2: 5'-GGCRAAKARRAARTAYCATTC-3'. Double-stranded polymerase chain reactions (PCRs) were performed in a 20-μl reaction volume with the following thermal cycles: 1 cycle at 94°C (3 min); 35 cycles at 94°C (30 s), 55°C (40 s), and 72°C (90 s); and 1 cycle at 72°C (10 min). Products were run on

Fig. 1. *Ophisaurus* with and without dorsal markings (A and B, respectively). The hatchling is uniform in the dorsal and black in the ventral coloration (C).

1.5% agarose gels in 1X TBE buffer to ensure that the lengths of certain fragments were correctly amplified. Blank controls were run each time to ensure that no contamination occurred during the PCR analyses.

PCR products were purified with a PCR Product Pre-Sequencing Kit (USB Corp.), and subsequently used as a template for the direct DNA sequencing reaction with a DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech). The same primers for PCR amplification were used for the sequencing reactions. In addition, 2 internal primers, PL2: 5'-ACAAACTAGGATTAGATACCC-3', and PH2: 5'-TATCGATTATAGGACAGGCTCC-3', were designed to facilitate the complete sequencing of the *12S rRNA* gene. Sequencing products were purified by ethanol precipitation and run on a MegaBACE 1000 automated DNA sequencer (Amersham Biosciences). Original signals were basecalled with a MegaBACE Sequence Analyzer 3.0 (Amersham Biosciences, 2001), and manually checked and modified using SEQUENCHER 4.0.5 (Gene Codes Corp., 1999). All sequences obtained in this study were repetitively checked in 2 directions. These sequences were also compared with those of other Squamata species to ensure the accuracy of the PCR amplifications. All haplotypes in this study were submitted to GenBank.

Sequence alignment and phylogenetic analyses

Sequences were assembled with the Pileup option in the GCG package (Wisconsin Package, vers. 10.1, Genetics Computer Group, Madison, WI), and modified by making manual adjustments. Pair-wise distances among individuals were calculated separately for the 2 gene fragments with Kimura's 2-parameter model (Kimura 1980). These 2 fragments were then combined for phylogenetic analyses. The same regions in an *Eumeces* skink (Kumazawa and Nishida 1999) were included to root the trees.

Construction of phylogenetic trees was performed using PAUP 4.0b10 (Swofford 2002). Maximum-likelihood (ML) was performed with the HKY85 (Hasegawa et al. 1985) distance model (unequal nucleotide composition and an unequal transition/transversions ratio). Heuristic searches were applied with 1000 bootstrap replicates performed to examine the robustness of each branch. Maximum-parsimony (MP) and Neighbor-joining (NJ) analyses were performed as well, with both using 2000 bootstrap replicates. The degree of gene flow among different OTUs was calculated

Table 1. A review of the literature summarizing 8 different characters described for species recognition between *Ophisaurus formosensis* and *O. harti*. The first 3 characters were proposed by Kishida (1930), and the others were proposed by later herpetologists. Some of them were repetitively applied in various references

Year	Author	Characters used								Scale definitions
		1	2	3	4	5	6	7	8	
1930	Kishida	˅	˅	˅						A
1956	Chen	˅								A
1956	Wang and Wang					˅				not defined
1969	Chen	˅								A
1970	Liu-Yu	˅			˅	˅	˅			B
1976	Wang and Liang				˅		˅	˅		not defined
1984	Chen and Yu				˅		˅			not defined
1987	Lue et al.	˅			˅		˅			not defined
1990	Lin and Cheng	˅			˅		˅			not defined
1990	Lue	˅					˅			not defined
1999	Lue et al.	˅			˅					not defined
1999	Zhao et al.	˅			˅				˅	A
2001	Shang	˅			˅					not defined

Characters: 1. Dorsal coloration; 2. Black dots on the head; 3. Shape of the occipital plate; 4. No. of longitudinal series of dorsal scales; 5. No. of scales separating nasal from rostral; 6. No. of scales separating frontonasal from rostral; 7. No. of scales separating frontonasal from nasal; and 8. No. of scales separating prefrontal from nasal. Scale definitions: A. Following Kishida (1930) with a single prefrontal before the frontal; B. Following Liu-Yu (1970) with a single frontonasal before the frontal.

using DnaSP 3.14 (Rozas and Rozas 1999).

Literature review and morphological comparisons

For a wide survey of morphological characters described for species recognition, we compared all literature describing the distinction between *O. formosensis* and *O. harti*. A summary of these references revealed that 8 characters have been applied for species identification by different authors. These 8 characters were organized in order by date of publication, and were recorded from the 19 *Ophisaurus* specimens in our collection. These characters were also evaluated in 5 additional formalin-fixed *Ophisaurus* individuals, not included in the molecular analyses. Finally, we carefully inspected the drawing of the holotype of *O. formosensis* (Kishida 1930). Lepidosis characters from the holotype were compared to our specimens and the literature.

RESULTS

Sequence characteristics

Five haplotypes of the mitochondrial *12S rRNA* gene were obtained from 5 *O. formosensis* individuals, and 7 were obtained from the 14 *O. harti*. The GenBank accession numbers of these haplotypes are listed in table 2. However, 3 of them are shared by the 2 species (AF380947, AF380948, and AF380949). The length of the mitochondrial *12S rRNA* gene is 947-948 bp in *O. formosensis* and *O. harti*, 951 bp in *O. gracilis* (AF380953), and 944 bp in *P. apodus* (AF380954). Ten variable sites were observed among haplotypes in Taiwan, including 7 transitions, 2 transversions, and 1 site at which a transition, transversion, and indel coexisted. No variable sites were observed in the adjacent 69-bp *tRNA-Val* among *O. formosensis* and *O. harti* individuals. The length of this fragment is 68 bp in *O. gracilis* and *P. apodus*.

The *cytochrome b* gene used in the analyses is 711 bp in length. Five haplotypes were obtained from *O. formosensis* and 7 from *O. harti*. Two haplotypes were shared by the 2 species (AF380956 and AF380963). Twenty-six variable sites exist among haplotypes in Taiwan, including 19 transitions, 6 transversions, and 1 at which both a transition and transversion occurred. The same gene fragments of *O. gracilis* (AF380964) and *P. apodus* (AF380965) were also obtained.

Phylogenetic analyses

The mean values and ranges for interspecific distances and intraspecific variations are listed in table 3. The minimum genetic distance between *O. formosensis* and *O. harti* was 0 due to the shared haplotypes. The mean value for the interspecific distances between these 2 species (0.0022 for *12S rRNA* and 0.0114 for *cytochrome b*) were far less than those between other species pairings (exceeding 0.08 for *12S rRNA* and 0.18 for *cytochrome b*), and even smaller than the mean intraspecific variations within *O. formosensis* (0.0033 and 0.0148 for the 2 respective gene fragments).

In the 1743 bases aligned for tree construction, 613 characters were variable, and 159 were parsimony-informative. However, only 23 characters were informative among *Ophisaurus* in Taiwan. The ML tree of this dataset is shown in

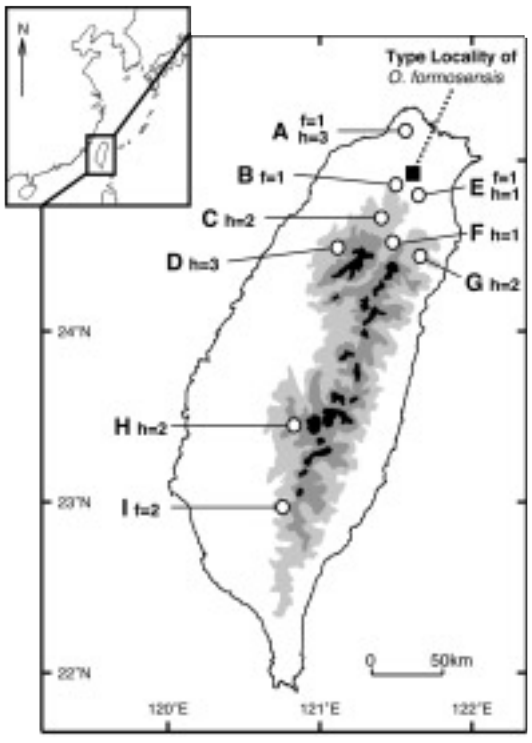

Fig. 2. Collecting sites of *Ophisaurus* species in Taiwan. A: Mt. Yangming, B: Sanhsia, C: Baling, D: Guanwu, E: Fushan, F: Datung, G: Nanao, H: Mt. Ali, and I: Shanping. The type locality of *O. formosensis*, Hinokiyama (=Kueishan), is denoted by the closed rectangle. Sampling numbers are noted on the map: "f" denotes *O. formosensis*, and "h" denotes *O. harti*. They are sympatrically distributed at sites A and E.

figure 3 with bootstrap values on the major branches. The MP tree and NJ tree reveal similar tree topologies with similar bootstrap values. All these criteria revealed 100% bootstrap support for the monophyletic clustering of specimens in Taiwan. Individuals of *O. formosensis* and *O. harti* were mixed in this clade, revealing no genetic differentiation between these 2 species. Gene flow calculations between these 2 species revealed an F_{st} value of 0.029 and an N_m value of 8.67, indicating a confluent gene pool among all individuals.

Literature review and morphological characters of collected specimens

Thirteen references were included in the literature review for species recognition (Table 1). A summary of the characters used for species identification includes the following.

1. Dorsal coloration: with blue markings in *O. harti* and without them in *O. formosensis*. This character is based on the original description of *O. formosensis* by Kishida (1930), and is widely applied for species recognition.
2. Black dots on the head: with dots in *O. harti* and without them in *O. formosensis* (Kishida 1930). This has never been applied since Kishida.
3. The shape of the occipital plate: more distinctly differentiated from other scales in *O. formosensis* than in *O. harti* (Kishida 1930). This has never been applied since Kishida.
4. The number of the longitudinal series of dorsal

Table 2. Sampling localities, morphological character states, and *12S rRNA/cytochrome b* accession numbers in GenBank of each *Ophisaurus* individual in this study. Definitions of the 8 morphological characters are listed in the text and table 1

			Morphological characters								Accession numbers	
Species	Number	Sample locality	1	2	3	4	5	6	7	8	*12S rRNA*	*Cytochrome b*
O. formosensis	F01	A: Mt. Yangming, Taipei	–	–	+	16	1	2	2	2	AF380948	AF380962
O. formosensis	F02	B: Sanhsia, Taipei Co.	–	+	+	16	1	2	2	2	AF380949	AF380963
O. formosensis	F03	E: Fushan, Yilan Co.	–	+	–	16	1	2	2	2	AF380947	AF380956
O. formosensis	F04	I: Shanping, Kaohsiung Co.	–	+	+	14	1	2	2	2	AF526566	AF526568
O. formosensis	F05	I: Shanping, Kaohsiung Co.	–	+	+	?	1	2	2	2	AF526567	AF526569
O. harti	H01	A: Mt. Yangming, Taipei	+	–	–	18	1	3	2	2	AF380949	AF380957
O. harti	H02	A: Mt. Yangming, Taipei	+	+	+	16	1	2	2	2	AF380948	AF380956
O. harti	H03	A: Mt. Yangming, Taipei	+	–	+	16	1	2	2	2	AF380949	AF380963
O. harti	H04	C: Baling, Taoyuan Co.	+	?	?	?	?	?	?	?	AF380947	AF380956
O. harti	H05	C: Baling, Taoyuan Co.	+	–	+	16	1	2	2	2	AF380949	AF380963
O. harti	H06	D: Guanwu, Miaoli Co.	+	–	+	16	1	2	2	2	AF380947	AF380956
O. harti	H07	D: Guanwu, Miaoli Co.	+	–	+	16	1	2	2	2	AF380947	AF380956
O. harti	H08	D: Guanwu, Miaoli Co.	+	–	+	16	1	2	2	2	AF380947	AF380956
O. harti	H09	E: Fushan, Yilan Co.	+	–	–	16	1	2	2	2	AF380947	AF380956
O. harti	H10	F: Datong, Yilan Co.	+	–	+	16	1	2	2	2	AF380947	AF380958
O. harti	H11	G: Nanao, Yilan Co.	+	–	+	16	1	2	2	2	AF380950	AF380959
O. harti	H12	G: Nanao, Yilan Co.	+	+	+	16	1	2	2	2	AF526565	AF380959
O. harti	H13	H: Mt. Ali, Chiayi Co.	+	?	?	?	?	?	?	?	AF380951	AF380960
O. harti	H14	H: Mt. Ali, Chiayi Co.	+	?	?	?	?	?	?	?	AF380952	AF380961
O. formosensis	F06	A: Mt. Yangming, Taipei	–	–	+	16	1	2	2	2		
O. harti	H15	A: Mt. Yangming, Taipei	+	–	–	16	1	2	2	2		
O. harti	H16	A: Mt. Yangming, Taipei	+	–	–	16	1	2	2	2	Formalin-fixed specimens	
O. harti	H17	A: Mt. Yangming, Taipei	+	–	+	16	1	2	2	2		
O. harti	H18	A: Mt. Yangming, Taipei	+	–	+	16	1	2	2	2		
O. formosensis	Kishida (1930)		–	–	+	?	1	2	2	2		
O. harti	Liu-Yu (1970)		+	?	+	16	0	2	2	2	Drawings in the references	
O. harti	Brygoo (1987)		+	?	–	?	?	2	?	?	(represented in Fig. 4)	
O. harti	Zhao et al. (1999)		+	?	+	16-18	1	2	2	2		
O. gracilis	Brygoo (1987)		?	?	+	?	1	6-7	3	3	Descriptions of *O. gracilis*	
O. gracilis	Zhao et al. (1999)		+	?	–	14	1	5	3	3		
O. gracilis	Yunnan, China (our collection)		+	–	–	14	1	6	3	3	AF380953	AF380964

"?" denotes missing information: indistinguishable from the specimen, indistinguishable from the original drawings, or not described in that reference. Formalin-fixed specimens were not used for molecular analyses.

scales: 14 in *O. formosensis* and 16-18 in *O. harti*. This was first proposed by Liu-Yu (1970) and has been applied 8 times.

5. The number of scales separating the nasal from the rostral: 1 in *O. formosensis* and 2 in *O. harti*. This was first proposed by Wang and Wang (1956) and has been applied twice.
6. The number of scales separating the frontonasal from the rostral: 1 in *O. formosensis* and 2 in *O. harti*. This was first proposed by Liu-Yu (1970) and has been applied 6 times.
7. The number of scales separating the frontonasal from the nasal: 1 in *O. formosensis* and 2 in *O. harti*. This was first proposed by Wang and Liang (1976) and has never been applied since then.
8. The number of scales separating the prefrontal from the nasal: 1 in *O. formosensis* and 2 in *O. harti*. This was first proposed by Zhao et al. (1999).

Of the 8 morphological characters used to distinguish *O. harti* and *O. formosensis*, 3 were used by Kishida in 1930, and 5 were proposed by later herpetologists. All of these characters were observed and measured in all 24 specimens (Table 2), except for some road-kill specimens too badly damaged to obtain their character state.

Except for 1 individual from Mt. Yangming (H01), which had an extra scale between the frontonasal and the rostral, all *Ophisaurus* in Taiwan had a similar number of scales in head lepidosis (characters 5-8). The number of the longitudinal series (character 4) of dorsal scales ranged between 14 and 16 in *O. formosensis*, and 16 and 18 in *O. harti*. Characters 2 and 3, which had not been applied since Kishida (1930), were irregular and added no resolution to species recognition (Table 2).

DISCUSSION

The presence of *Ophisaurus* in Taiwan was first reported by Van Denburgh (1909) based on a single specimen collected by the Canadian missionary Dr. George Leslie Mackay (1844-1901). This specimen was classified as *O. harti*, with speculation that it was a distinct species. Following the statement of Van Denburgh, Stejneger (1910) classified the specific name as "undecided" in his list of the Taiwanese herpetofauna. But later after receiving and comparing specimens from Foochow (=Fuzhou), Fokien (=Fujian), China (near the type locality of *O. harti*, Guadun, Fujian) and Taiwan, he claimed: "I therefore have no doubt that the Formosan specimens are correctly identified as *Ophisaurus harti*." (Stejneger 1919).

Table 3. Mean values and range of interspecific genetic distances and intraspecific variations calculated with Kimura's 2-parameter method: (A) complete *12S rRNA* gene; and (B) partial *cytochrome b* gene

(A) 12S rRNA

	O. formosensis	*O. harti*	*O. gracilis*	*P. apodus*
O. formosensis	0.0033 0.0000-0.0059	0.0022 0.0000-0.0079	0.0875 0.0876-0.0886	0.0966 0.0937-0.1018
O. harti		0.0012 0.0000-0.0040	0.0881 0.0854-0.0909	0.0951 0.0937-0.0983
O. gracilis			—	0.1180 0.1174-0.1185
P. apodus				—

(B) cytochrome *b*

	O. formosensis	*O. harti*	*O. gracilis*	*P. apodus*
O. formosensis	0.0148 0.0014-0.0216	0.0114 0.0000-0.0246	0.1845 0.1768-0.1859	0.2222 0.2170-0.2251
O. harti		0.0090 0.0000-0.0231	0.1805 0.1768-0.1838	0.2209 0.2170-0.2229
O. gracilis			—	0.2108
P. apodus				—

Ophisaurus formosensis was first described by Kishida in 1930. The holotype was an adult female collected from Hinokiyama (=Mt. Kueishan), Taihoku (=Taipei), northern Taiwan (indicated in Fig. 2). Based on Kishida's description, the major differences between *O. formosensis* and *O. harti* were 1) a lack of dorsal blue spots, 2) a lack of black dots on the head, and 3) a fan-shaped and more distinctly differentiated azygous occipital plate. The last 2 characters, however, were never mentioned again since Kishida. Based on our examination, the appearance of these 2 characters is irregular and unstable among *Ophisaurus* individuals (Table 2). These 2 characters are obviously not appropriate for species recognition.

Since 1930, 5 new identifications have been proposed by other herpetologists (Table 1). The addition of these characters complicated the situation. The most frequently applied character has been the number of dorsal scales (character 4), which appeared 8 times in the literature after 1930, suggesting 14 rows in *O. formosensis* and 16-18 in *O. harti*. Among our 6 individuals of *O. formosensis*, only 1 had 14 rows of dorsal scales (F04, an adult female from location I). The other 5 specimens, like the majority of *O. harti* specimens, had 16 dorsal rows. Kishida (1930) did not provide the row number of *O. formosensis* in his description of the holotype, suggesting that the characteristic of 14 dorsal rows is not appropriate for *O. formosensis*. Individuals with 14 dorsal rows indeed exist, but may account for only a small proportion.

We noticed that specimens fixed in formalin or alcohol are sometimes altered by the preservation. Individuals of *Ophisaurus* have a pair of lateral grooves on either side of the body. With specimens in poor condition (e.g., hungry or poorly nourished), these grooves may cover 1 to 2 rows of dorsal scales on each side, affecting the accuracy of the count. This alteration is especially prominent in specimens dehydrated by alcohol or refrigeration. This character should be carefully evaluated whenever it is applied.

The other 4 characters (Nos. 5-8) focus on the lepidosis of the head. One should note that Liu-Yu was the first to apply an abnormal scale definition different from the usual. *Ophisaurus* species have only a single prefrontal scale (Kishida 1930, Zhao et al. 1999), as opposed to skinks, which usually have a pair of prefrontal scales connecting to each other in front of the frontal. According to the description by Liu-Yu (1970), the "frontonasal" scale by her definition is the same as the "prefrontal" scale in Kishida's drawing. Since none of the subsequent literature mentioning this scale precisely defines the position of the "frontonasal" in *Ophisaurus*, we inferred that most of them followed Liu-Yu's definition and indicate the same as the "prefrontal". Hence, character 7 proposed by Wang and Liang (1976) (frontonasal to nasal) and character 8 proposed by Zhao (1999) (prefrontal to nasal) probably indicate the same character.

The most widely applied character on the head is the number of scales separating the frontonasal from the rostral (character 6). This character has been applied 6 times since being proposed by Liu-Yu (1970). According to their descriptions, there should be only 1 scale separating the frontonasal from the rostral in *O. formosensis*. However, all of the *Ophisaurus* specimens in our collections have 2 scales, except for a single *O. harti* specimen with 3 scales (H01 from Mt.

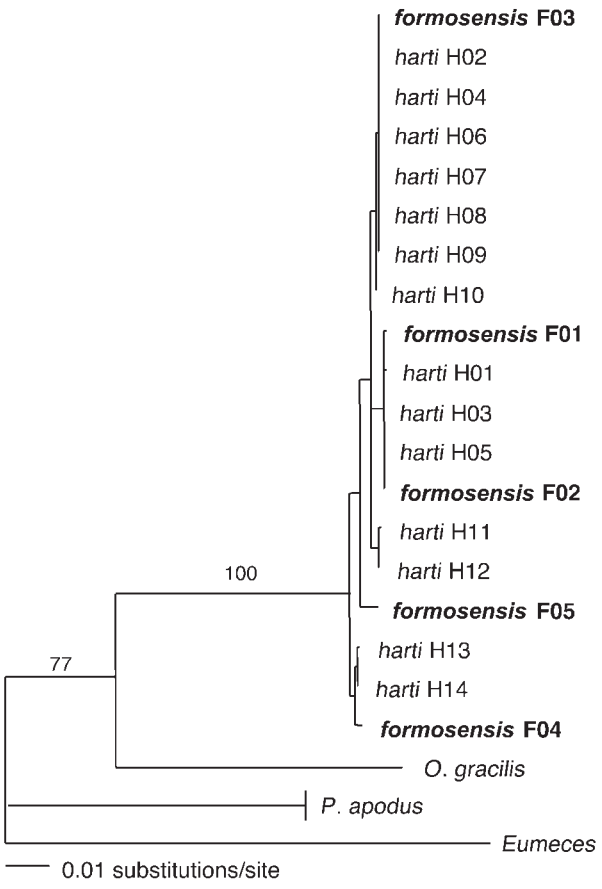

Fig. 3. Phylogenetic tree of *Ophisaurus* in Taiwan constructed using PAUP 4.0b10 with the data combining *12S rRNA* and *cytochrome b* sequences. This tree was constructed using maximum-likelihood criterion with the HKY85 model and 1000 bootstrap replicates.

Yangming). A similar pattern was found for characters 5, 7, and 8 for which no variation was observed among our *Ophisaurus* specimens (Table 2). These characters are useful in distinguishing *O. gracilis* from *O. harti*, but could not distinguish between the 2 species in Taiwan.

What should a "typical" *O. formosensis* look like? Inspecting the original drawing of *O. formosensis* by Kishida (1930), we found that the holotype of *O. formosensis* (Fig. 4A-B) is precisely identical to *O. harti* in head characters: 1 scale separating the nasal from the rostral; 2 scales separating the prefrontal from the rostral; and 2 scales separating the prefrontal from the nasal. It is obvious that *O. formosensis* and *O. harti* should not be distinguished based on these head lepidosis characters. Characteristics proposed by later herpetologists did not fit the original description of the holotype, and this is probably how the taxonomic controversy arose.

In addition, analyses of the 1743-bp mitochondrial sequences do not support genetic differentiation between these 2 color morphs. Shared haplotypes were commonly found among individuals in northern Taiwan, leading to the "interspecific" distance sometimes being zero. The genetic distance between northern and southern individuals was even greater than that between different color morphs, indicating that the distance factor contributed more to their genetic variation than did coloration. In addition, the results of phylogenetic construction revealed no clustering of similar color morphs (Fig. 3). The low F_{st} (0.029) and the high N_m (8.67) values indicate confluent gene flow with no reproductive isolation. We conclude that there should be only 1 *Ophisaurus* species but 2 color morphs in Taiwan.

Possible explanations of dichromatism in reptiles include geographic variation, mature-immature variation, and between-sex variation. The first hypothesis is excluded due to their sympatric distribution. The second hypothesis is possible because hatchlings of *O. harti* (Fig. 1C) are indeed uniform in dorsal coloration and lack blue markings (Lin and Cheng 1990, Huang and Chou 1993). However, this hypothesis does not explain the entire story because all of our 6 specimens without markings had mature body sizes, including a female which laid 6 eggs while in captivity. We suspect that sexual dichromatism is a probable explanation: females tend to show uniform dorsal coloration more often than males. This deduction

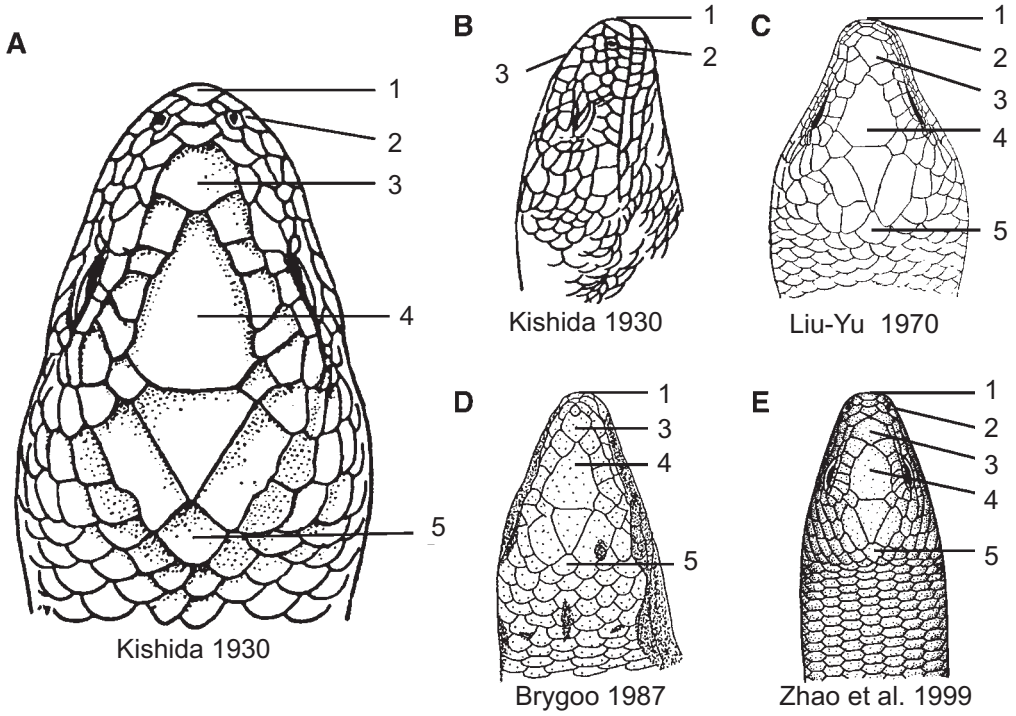

Fig. 4. Dorsal view (A) and lateral view (B) of the holotype of *Ophisaurus formosensis* copied from the original drawing of Kishida (1930), and 3 dorsal versions of *O. harti*: (C) Liu-Yu 1970, (D) Brygoo 1987, and (E) Zhao et al. 1999. Examination of these figures indicates no differences in lepidosis characters of the head between *O. formosensis* and *O. harti*. Scales are defined as follows: 1: rostral, 2: nasal, 3: prefrontal (or frontonasal by Liu-Yu's definition), 4: frontal, and 5: occipital.

is based on a limited and preliminary observation that 2 of the specimens without markings were revealed to be female, and several of the individuals with blue markings were revealed to be male. This hypothesis also explains the much rarer record of individuals without markings due to the lower mobility of females. Nevertheless, most specimens in our current collection were too badly damaged to determine their sex. More specimens are needed to verify this hypothesis in the future.

According to comparisons among the specimens and the literature, both color morphs of *Ophisaurus* in Taiwan are very similar, or even identical to *O. harti* in morphology. We conclude that *O. formosensis* should be a synonym of *O. harti*. The scientific name of *formosensis* will be valid only if researches in the future determine that distinctive differences exist between populations in Taiwan and mainland China. In the current situation, we should tentatively use the senior synonym, *O. harti*, to indicate the legless lizard which occurs in Taiwan. In the meanwhile, it is still critical to examine specimens from mainland China in further studies, especially using molecular analyses. Whether the dichromatism of *O. harti* occurs in other populations also needs to be investigated.

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