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A NEW SPECIES OF *CUORA* (REPTILIA: TESTUDINES: EMYDIDAE) FROM THE RYUKYU ISLANDS

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Abstract.—A new species of Asian box turtle, *Cuora evelynae,* from the Ryukyu Islands is described and compared with *C. flavomarginata* from Taiwan and southern China. Recognition is based on its pattern of very large light colored pleural blotches (length of blotch at its medial height 49–72% of medial length of pleural) that at their dorsal border coalesce to form lateral stripes in 52% of adults examined, its large light brown plastral blotch which is indented at the bridge, and the usual presence of less than 10 rows of large scales on the anterior surface of the forelimbs. A discriminant function is given that separates *C. evelynae* from *C. flavomarginata.* Geological history of the Ryukyu Islands is discussed, in view of speciation of *C. evelynae.*

The yellow-margined box turtle, *Cuora flavomarginata* (Gray, 1863) occurs in southern China, Taiwan, and the Ryukyu Islands (Iverson 1986). Even though these localities have been long isolated (Inger 1947), no consistent differentiation of these turtle populations has been reported previously.

Hsu (1930) described a subspecies, *Cuora f. sinensis,* from Tungting Lake, Hunan, China, as differing from the nominate Taiwanese population on the basis of having the anterior border of the plastron obtusely emarginate, each plastral scute with deeply cut parallel lines, each marginal with its posterior angle slightly overlapping the next with the degree of overlap most pronounced in the posterior third of the marginals so that the posterior carapacial rim is somewhat serrated, a small notch between the anal scutes, and a much shorter tail. Pope (1935) commented that with the exception of the longer tail (which may be sexual dimorphic), all the other characters used by Hsu are either common variants expected in most emydid turtles or differences generally correlated with age, but Pope thought that until direct comparison between Chinese and Taiwanese *C. flavomarginata* could be made, it was best to consider the two populations as distinct subspecies. Tanaka & Sato (1983) have referred to the turtles on the Ryukyu Island of Iriomote as *Cuora f. flavomarginata* indicating an affinity with the population on Taiwan. However, Fang (1934) had critically compared specimens from Taiwan and the Ryukyu Islands with Hsu's diagnostic characters and found that the mainland Chinese turtles could not be differentiated from the insular populations.

Recent examination and comparison of *C. flavomarginata* from these three populations has shown that turtles from the Ryukyu Islands can be distinguished from those of the other two populations and represent an undescribed species.

Methods and materials.—Sixty-six turtles were examined (Ryukyu Islands, 38; Taiwan, 14; China, 14). Sexes were determined by the characters given by Ernst & Barbour (1989). Straight-line measurements of each specimen were taken with dial calipers accurate to 0.1 mm. Variables included: the greatest carapace length, carapace width and depth at the level of the seam separating vertebrals 2 and 3, marginal width (the dif-

ference between the carapacial width and the width across the pleurals taken between the points of juncture of the marginals and pleurals at the level of the seam between vertebrals 2 and 3), greatest plastron length, greatest width and length of both plastral lobes, least bridge length, greatest width and length of the cervical scute and all vertebrals and medial seam lengths of all plastral scutes (Gul., Hum., Pect., Abd., Fem., An.). Careful notes and drawings were made of head, neck, limb, carapacial and plastral patterns. Colors were recorded from living turtles and color transparencies. Shell proportions were expressed as ratios of one measurement to another. Several ratios proved useful in our description (abbreviations used in text are given in parentheses): width/length of cervical scute (W/L CS), width/length of designated vertebrals (W/L 1st, V W/L 2nd V, etc.), marginal width/carapacial width (MW/CW), marginal width/carapacial length (MW/CL), carapacial width/carapacial length (CW/CL), carapacial depth/carapacial length (D/CL), carapacial depth/carapacial width (D/CW), length of light-colored blotch at its medial heights on pleural scute 2/total length of pleural scute 2 at the same point (PBL/PSL), plastral length/carapacial length (PL/CL), bridge length/plastral length (B/PL), bridge length/carapacial length (B/CL), length of the anterior plastral lobe/plastral length (APL/PL), width of anterior plastral lobe/plastral length (APW/PL), width of anterior plastral lobe/length of anterior plastral lobe (APW/APL), length of posterior plastral lobe/plastral length (PPL/PL), width of posterior plastral lobe/plastral length (PPW/PL), and width of posterior plastral lobe/length of posterior plastral lobe (PPW/PPL). The number of rows of large scales at the lateral edge of the antebrachium between the claw of digit 5 and the first horizontal skin fold proximal to the elbow (presented in text as FLSR) was recorded.

Statistical techniques were executed using SYSTAT (Wilkinson 1986) and STATGRAPHICS (STSC 1986). Males and females were combined for analysis since statistically significant size differences were not detected ($\bar{x}$ male CL, 137.6 mm; $\bar{x}$ female CL, 143.3 mm: ANOVA, F = 0.98; df = 1,39; $P = 0.33$). Juveniles (23) were included in all analyses due to small total sample size (66). Males, females and juveniles were evenly distributed between population samples. Variables were transformed for parametric analysis as follows: those based on proportions, such as the interanal seam length (An) and PBL/PSL, were arcsine square root transformed, FLSR was square root transformed, and natural logarithms were taken of all others to reduce variance (Lewontin 1966, Moriarty 1977). Principal components analysis (PCA) was used as a data reduction technique to identify orthogonal factors and their important variables. Interanal seam length (An) was excluded from this step because it had previously been identified to vary independently of population. Variables with high factor loadings were used in subsequent analyses as were factor scores (Kachigan 1986). Following PCA, differences among populations were tested using multivariate analysis of variance (MANOVA) as suggested by Willig et al. (1986). Following identification of differences among these putative populations, a three-group discriminant function analysis was conducted using the raw variables selected with PCA. Discriminant scores were calculated by multiplying these variables by their associated unstandardized canonical coefficients and summing the products. Each specimen was then plotted along the axes providing maximal separation of the a priori groups. Levels of significance were set a priori at alpha = 0.05.

Specimens from the following collections were examined: American Museum of Natural History (AMNH), California Academy of Sciences (CAS), Field Museum of Natural History (FMNH), George Mason University (GMU), Museum of Comparative Zo-

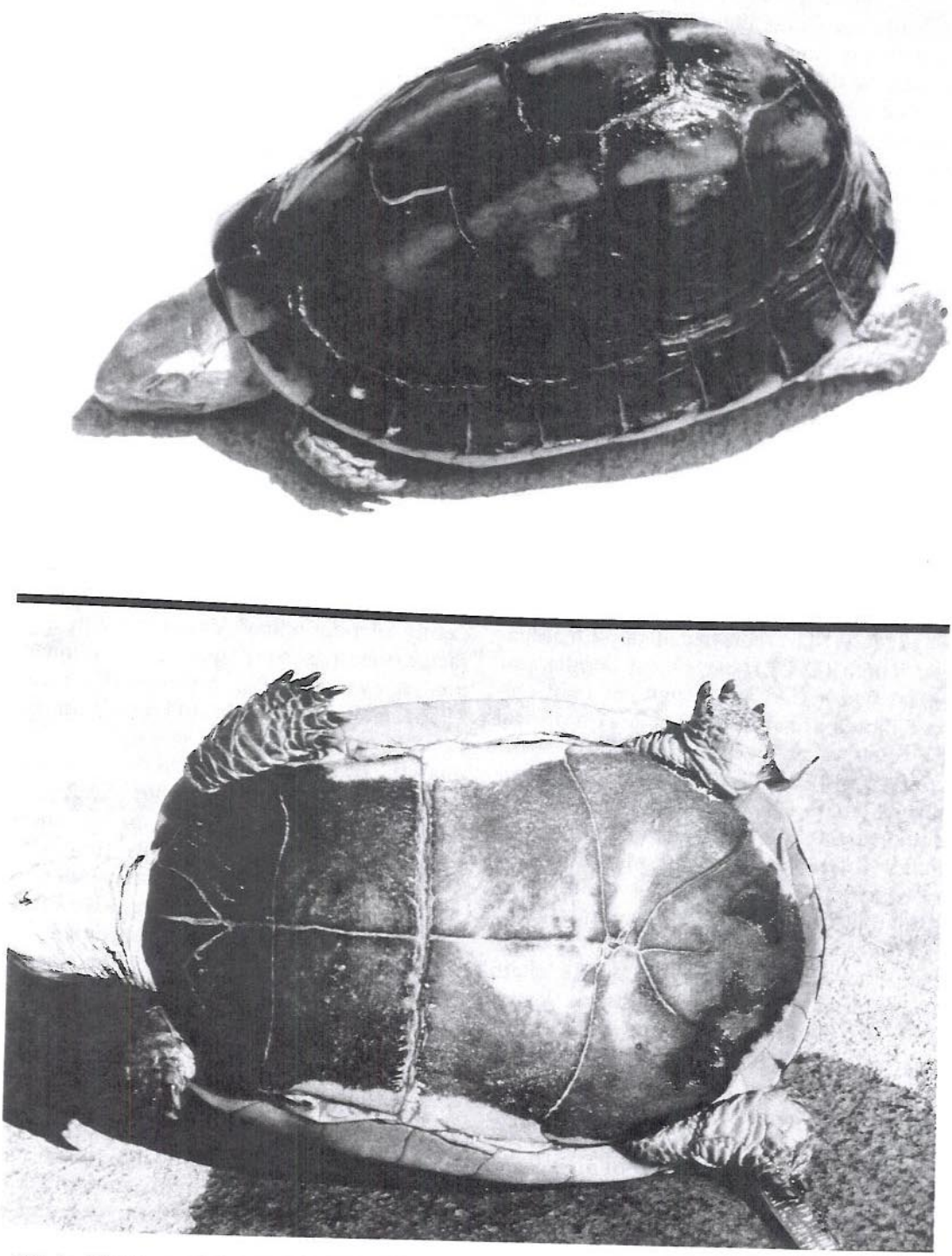

Fig. 1. Carapace and plastron of *Cuora evelynae*, new species (AMNH 50804).

Table 1.—Summary statistics for significant variables by populations. Means (mm) are followed by one standard error in parentheses. Probabilities are given for univariate F-tests between localities. Refer to text for abbreviations.

Variable	Population (n)			Probability
	Ryukyu Islands (35)	Taiwan (13)	China (14)	
CW	90.6 (3.2)	74.9 (6.7)	92.0 (5.7)	0.03
APW	63.7 (2.5)	50.8 (5.3)	67.2 (4.5)	0.01
PPW	71.4 (3.0)	57.0 (6.3)	76.3 (5.3)	0.02
Gul	19.7 (0.7)	16.4 (1.7)	19.6 (1.2)	0.05
Hum	6.0 (0.4)	6.6 (0.6)	8.8 (0.8)	0.01
Abd	32.3 (1.5)	24.4 (2.8)	28.9 (2.4)	0.02
FLSR	8.8 (0.2)*	10.8 (0.6)	8.9 (0.2)	<0.001
PBL/PSL (%)	64.6 (0.5)	34.2 (1.0)	35.3 (0.4)	<0.001

* Includes four additional specimens not included in other statistical analyses.

ology, Harvard University (MCZ), Naturhistorisches Museum Wien, Vienna (NHMW), United States National Museum of Natural History (USNM), William P. McCord, Hopewell Junction, New York, personal collection (WPM), and William H. Randel, Hatboro, Pennsylvania, personal collection (WHR).

Results and discussion.—Turtles from the Ryukyu Islands showed two pattern differences from the other two populations. The pleural blotch in these specimens was larger (PBL/PSL; $\bar{x}$ = 65%) than those of turtles from Taiwan (34%) and mainland China (35%). The pleural blotch accounted for significantly different proportions of the pleural scute length (PBL/PSL) between populations (ANOVA; F = 703.08; df = 2,59; P < 0.0001). In 14 of 27 adults (52%) from the Ryukyu's the blotches were so large as to coalesce dorsally, forming two lateral light longitudinal stripes in addition to the normal medial stripe (Fig. 1). No specimens from either Taiwan or China had lateral stripes. The shape and coloration of the dark medial blotch on the plastron also varied (Fig. 1). Turtles from Ryukyu usually had a large, hourglass-shaped blotch, indented at the bridge, which was light brown in color; those from the other two populations had large dark brown to black blotches with straight sides or only a very shallow indentation at the bridge. Summary statistics and univariate test probabilities between populations are given for all significant variables in Table 1. Eight variables, about six more than expected at alpha = 0.05, show significant differences among populations.

The first three factors generated by PCA for 28 variables accounted for 93 percent of the total variance (Factor I—84%, eigenvalue = 23.49, Factor II—6%, eigenvalue = 1.65, Factor III—3%, eigenvalue = 0.96). The highest loadings in Factor I are all size related mensural variables. PPL had the highest loading in Factor I (0.997). The proportion of pleural blotch coloration (PBL) had the highest loading in Factor II (0.790). FLSR had a very high negative loading (−0.729) in Factor II. MW had the highest loading in Factor III (0.920). All other variables were highly correlated with these four and add little to the analysis, so they were not analyzed further. Factor scores for Factor I did not differ significantly between populations (ANOVA; F = 2.45; df = 2,50; P = 0.10), nor did scores for Factor III (ANOVA; F = 0.46; df = 2,50; P = 0.64). However, Factor II scores did (ANOVA; F = 55.85; df = 2,50; P < 0.001). Differences among populations were suggested by MANOVA when all three sets of factor

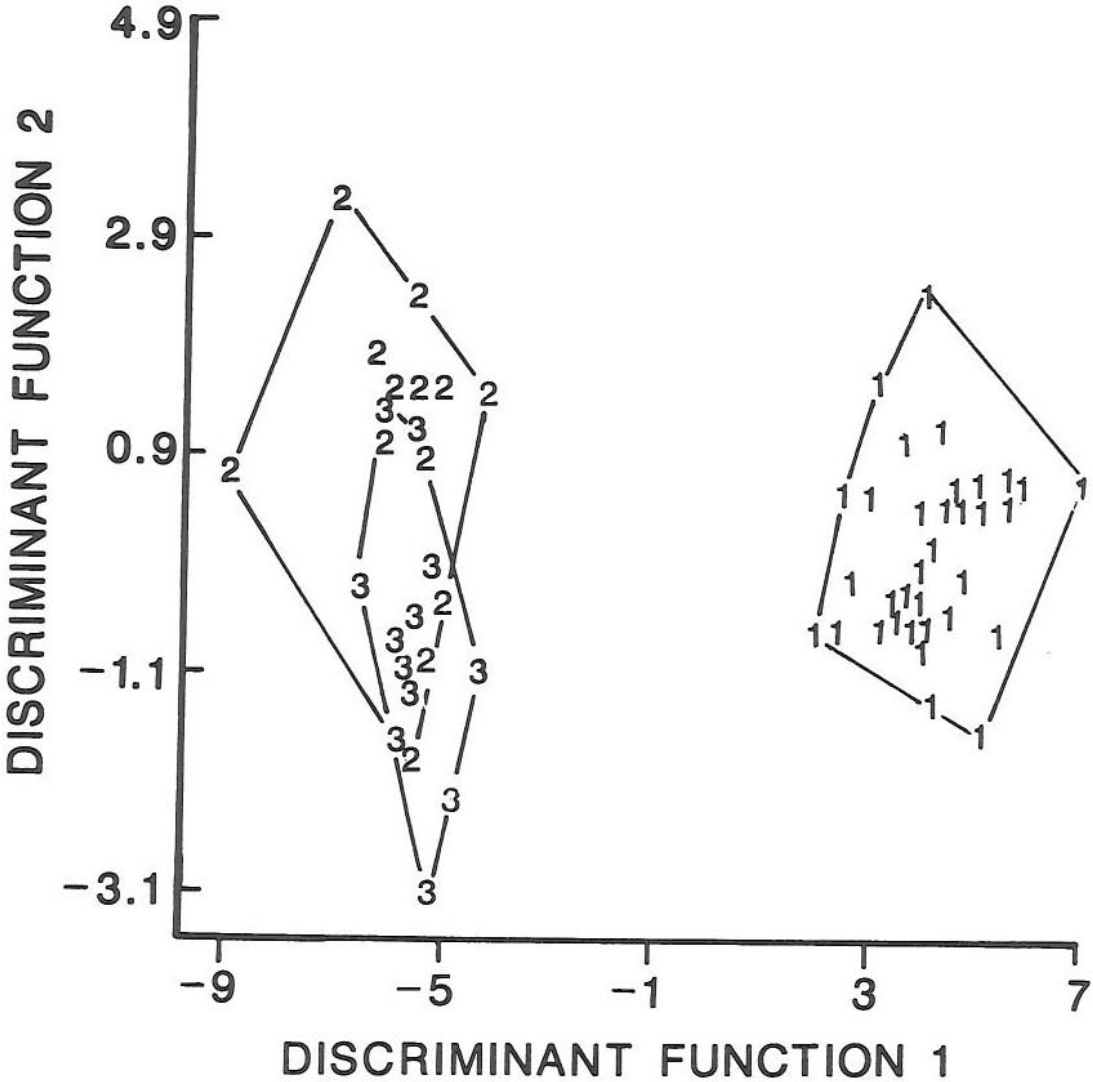

Fig. 2. Discriminant function analysis of turtle populations from the Ryukyu Islands(1), Taiwan(2), and southern China(3) for the characters PPL, PBL/PSL, MW, and FLSR. Polygons define extreme values of each cluster. Numbers represent individual turtles.

scores were included (Wilks' Lambda = 0.23; F = 17.76; df = 6,96; $P < 0.001$). Univariate F-tests for the highest loading variable in each factor were as follows: Factor I—PPL (ANOVA; F = 2.83; df = 2,59; $P = 0.07$), Factor II—PBL (ANOVA; F = 703.08; df = 2,59; $P < 0.001$), Factor III—MW (ANOVA; F = 0.92; df = 2,59; $P = 0.40$). Differences were again demonstrated using MANOVA for these three variables (Wilks' Lambda = 0.04; F = 82.64; df = 6,114; $P < 0.001$).

FLSR differed significantly between populations (ANOVA; F = 9.07; df = 2,56; $P < 0.001$). Specimens from Taiwan had a mean of 10.8 while those from the Ryukyu Islands and China had means of 8.8 and 8.9 respectively.

Variables with high loadings (PPL, PBL, FLSR, MW) were entered into a discrimi-

nant function analysis and each specimen was classified into a predicted population. The first and second discriminant functions provide significant ($P < 0.001$) discrimination between populations (Fig. 2). The function correctly classified 100% of the Ryukyu specimens, 92% of those from mainland China, and 77% of the specimens from Taiwan. Discriminant scores (DS) are > -1 for specimens from the Ryukyus and < -1 for specimens of *C. flavomarginata* from China and Taiwan based on the following discriminant function: DS = −18.6013 + 33.7378(PBL/PSL) + 0.02580(FLSR) + 0.01970(MW) + 0.00960(PPL). This function correctly classified all specimens of both species.

Although closely related to *C. flavomarginata,* the turtle population in the Ryukyu's can be distinguished by the combination of characters listed above. Since it is allopatric, and has apparently been isolated for more than a million years (see discussion below), we believe it to be at least an incipient species and have treated it as such, rather than as a subspecies of *C. flavomarginata.*

Cuora evelynae, new species
Fig. 1

Holotype.—CAS 26113, adult male; Ishigaki Shima, Ryukyu Islands, Japan; Victor Kuhne, 5–11 May 1910.

Paratypes.—CAS 21026-21029, 26102-26112, 26801 (five adult males, eleven adult females); AMNH 50804 (adult female); MCZ 56064 (adult male); USNM 34076-34079 (adult male, adult female, two juveniles) from the type-locality.

Diagnosis.—A domed species of *Cuora* with a yellow head; a dark brown to black carapace with a large yellow to light brown blotch which at its medial height extends 49–72% ($\bar{x} = 64.6$) across the middle of each pleural, and at its dorsal border coalesces to form lateral stripes in 52% of adults; a large pale brown plastral blotch, which is indented at the bridge; the plastron lacking a medial posterior notch; most adults with at least a partially obliterated interanal seam, and usually less than 10 ($\bar{x} = 8.8$) rows of large scales on the anterior surface of the foreleg (a character shared with the mainland Chinese population).

Description (from all specimens examined).—Carapace length to 164 mm (males 159, females 164), elliptical, domed (D/CL 0.41–0.52, $\bar{x} = 0.468$; D/CW 0.57–0.71, $\bar{x} = 0.648$; CW/CL 0.67–0.80, $\bar{x} = 0.725$); widest at marginals 8, highest at posterior of vertebral 2 or vertebral 3. Carapace sides straight, anterior marginals flared, posterior marginals at best only slightly serrated with no medial notch. Marginals over bridge downturned (MW 6.4–18.7 mm, $\bar{x} = 10.86$; MW/CW .056–.16, $\bar{x} = 0.108$; MW/CL 0.04–0.12, $\bar{x} = 0.078$). Marginals large, and approximately the same width throughout. Carapacial scutes in young individuals are rugose because of growth annuli; those of older turtles are worn smooth. Cervical scute rectangular to triangular, longer than wide (W/LCS 0.46–0.95, $\bar{x} = 0.782$). Vertebrals 3–5 wider than long, vertebral 2 usually longer than wide (79% of adults) and vertebral 1 may be either wider than long (53% of adults) or longer than wide (47% of adults). Vertebrals 4 and 5 widest, vertebral 5 posteriorly flared, vertebral 1 slightly flared anteriorly and only contacting marginal 1. Three low longitudinal keels present; medial keel most pronounced and extending along all 5 vertebrals, lateral keels extend from posterior of pleural 1 through pleural 4 and may disappear with age. Color black to dark brown; rim of marginals yellow. Medial yellow stripe always present. Areolae of pleurals and vertebrals yellow or light brown. Light areolae of pleurals large (PBL/PSL 0.49–0.72, $\bar{x} = 0.646$), and tend to coalesce at their dorsal borders to form lateral light stripes in 52% of adults (Fig. 1). Undersides of marginals yellow.

Plastron length to 160 mm (males 154, females 160), shorter than carapace in 23(85%) specimens (PL/CL 0.93–1.01, $\bar{x} =$

0.973), movable hinge between the pectoral and abdominal scutes. Posterior lobe longer and wider than anterior lobe (APL/PL 0.40–0.44, $\bar{x}$ = 0.420; PPL/PL 0.57–0.61, $\bar{x}$ = 0.586; APW/PL 0.49–0.57, $\bar{x}$ = 0.524; PPW/PL 0.55–0.62, $\bar{x}$ = 0.592; APW/CW 0.67–0.75, $\bar{x}$ = 0.712; PPW/CW 0.75–0.87, $\bar{x}$ = 0.800). Anterior lobe rounded in front; posterior lobe rounded in rear, usually without a medial notch. Bridge moderate (B/CL 0.27–0.37, $\bar{x}$ = 0.311; B/PL 0.28–0.39, $\bar{x}$ = 0.318); axillary and inguinal scutes generally lacking, or small. Average plastral formula Abd. > An. > Pect. > Gul. > Fem. > Hum.; 14 (40%) had this formula, but 4 other formulae occurred; 23 (66%) had Abd. > An., 11 (31%) had An. > Abd., 21 (60%) had Fem. > Hum., 14 (40%) had Hum. > Fem. Intergular and interanal seams often obliterated in large individuals. Plastron and bridge yellow, with a large pale brown medial blotch occurring on all scutes and indented at the bridge (Fig. 1).

Head narrow, snout slightly projecting; upper jaw with slight medial hook. Dorsally, the head is lemon-yellow to olive. Laterally behind the orbit and jaws, is a large yellow blotch that encloses the tympanum. This blotch is dark bordered dorsally. A second smaller yellow blotch lies dorsally and behind the first, and extends across the top of the head to touch a similar blotch from the other side just in front of the cervical skin fold. Jaws and chin immaculate yellow. Neck yellow to olive with no pattern.

Digits partially webbed. Forelimbs with large scales (6–11 rows, $\bar{x}$ = 8.8), 29 of 34 individuals (85.3%) had less than 10 rows; outer surface olive brown or yellow to reddish-brown, inner surface and sockets yellow. Hindlimbs with smaller scales, colored similar to forelimbs; hindfoot olive to brown with large scales at heel and ankle. Tail yellow dorsally and bordered by two olive or brown stripes, tip yellow.

Males with moderately concave plastra, and longer, thicker tails with the vent beyond the carapacial rim. Females with flat plastra and smaller tails with the vent beneath the posterior marginals.

Cuora evelynae is known only from the islands of Iriomote, Ishigaki, and Okinawa (Iverson 1986); the Okinawa specimen (MCZ 55838) may represent an escaped captive. These turtles probably originated from mainland China or Taiwan at a time when the Ryukyus were connected by a land bridge, or only separated by shallow water. Subsequent submergence of the land bridge isolated the Ryukyu population thus allowing for speciation to occur.

Based on the reports of Yabe & Aoki (1923), Yabe (1929a, b), and Hanzawa (1935), Inger (1947) has presented a summary of the geology of these islands. The Ryukyu cordillera arose in the late Permian or early Mesozoic Era. It seems to have broken into several mountain masses between the Permian and upper Eocene. After the Eocene there have been many fluctuations in sea level great enough to have altered radically the area available for use by terrestrial or semi-terrestrial animals such as *C. evelynae*. From the upper Eocene through the lower Oligocene, Iriomote and Ishigaki were probably submerged (Yabe & Aoki 1923, Hanzawa 1935), but the islands emerged again during the middle and upper Oligocene. Three additional submergences took place, finally ending in the middle Pleistocene, during which at least the highest points of Iriomote and Ishigaki were above sea level. Between these latter submergences, Iriomote and Ishigaki probably had direct land connections with China through Taiwan, and it was then that the parent stock of *C. evelynae* may have reached the islands. However, the possibility of turtles rafting from Taiwan on the Kuroshio Current can not be excluded (Lovich et al. 1985). Final inundation of this land bridge during the early to middle Pleistocene, about 1.5 million years ago, presumably isolated the turtles on the islands and subsequently

separated Iriomote and Ishigaki. Inger (1947) proposed a similar scenario to explain present variation in the amphibian fauna of the Ryukyus, which also shows strong Oriental affinities.

Ye (1985) summarized the fossil record of *Cuora* in China, Taiwan and Japan, emphasizing specimens of *C. flavomarginata*. The earliest records for the genus are from the late Miocene (approximately 8 million years ago) of Yunnan, and Pleistocene remains are known from Kyushu and Honshu, Japan. This raises the possibility that the Ryukyu Islands population of *C. evelynae* may have reached there by island hopping along the Japanese archipelago. However, since no fossils of the genus are known from Korea and no living species of *Cuora* reaches there, it is more likely that movement was in the opposite direction and that the Kyushu and Honshu turtles came from mainland China by way of Taiwan and the Ryukyus.

Other material.—*Cuora evelynae*: CAS 21015-24, 26114-15; GMU 730-31; MCZ 7997, 55838. *Cuora flavomarginata*: AMNH 110181; CAS 10834-40; FMNH 121225-26, 127324, 216515; GMU 975; NHMW 29515, 29518; USNM 140825; WPM 1-5(live); WHR 1-7(live).

Etymology.—The genitive noun used as the specific epithet honors Dr. Ernst's wife Evelyn for her years of encouragement and help, and for her contributions to the study of turtle helminths.

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