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Phylogenetic Relationships of the Asian Box Turtles of the Genus *Cuora* sensu lato (Reptilia: Bataguridae) Inferred from Mitochondrial DNA Sequences

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ABSTRACT—Phylogenetic relationships of the genus *Cuora* sensu lato (*Cuora* sensu stricto and *Cistoclemmys*) and other testudinoid genera were inferred from variations in 882 base positions of mitochondrial 12S and 16S rRNA genes. Results yielded a robust support to the monophyly of a group (*Cuora* group) consisting of *Cuora* sensu lato and the monotypic *Pyxidea*. Within the *Cuora* group, the continental *Cuora* (sensu stricto) and the two subspecies of *Ci. flavomarginata* constituted two well-supported monophyletic groups. Distinctly small interspecific genetic distances in the former groups suggested that in the continent speciations in *Cuora* took place much later than the primary divergences in the *Cuora* group, or speciations in other related genera, such as *Mauremys.* Our analyses failed to provide a substantial support to the monophyly of any other combinations of taxa within the *Cuora* group, including *Cuora* in broad and strict senses, and *Cistoclemmys* as consisting of *Ci. galbinifrons* and *Ci. flavomarginata*. Besides these, our results also suggested the non-monophyly for the Batagurinae and the Geoemydinae, and sister relationships of the Bataguridae with Testudinidae rather than with the Emydidae.

Key words: Bataguridae, Geoemydinae, *Cuora, Cistoclemmys, Pyxidea*

INTRODUCTION

The Bataguridae is the largest and phenotypically most divergent family in the order Testudines, and is distributed mainly from the temperate to tropical Asia but also in southwestern Europe, northern Africa and Latin America (Iverson, 1992). This family consists of two subfamilies: Batagurinae and Geoemydinae (Gaffney and Meylan, 1988). The former accommodates 22 extant species of 12 genera (David, 1994; McCord and Iverson, 1994), whereas the latter 43 species of 13 genera (David 1994; McCord *et al*., 1995, 2000; Fritz and Wischuf, 1997; Fritz *et al*., 1997; McCord, 1997; Wink *et al*., 2000; Parham *et al*., 2001; Yasukawa *et al*., 2001).

Within the Bataguridae, kinetic plastron with a hinge between the pectoral and the abdominal is observed in five geoemydine genera: *Cistoclemmys* Gray, 1863 (type species: *Ci. flavomarginata*), *Cuora* Gray, 1855 (type species: *Cu. amboinensis*), *Cyclemys* Bell, 1834 (type species: *Cy. dentata*), *Notochelys* Gray, 1863 (monotypic with *N. platynota*), and *Pyxidea* Gray, 1863 (monotypic with *P. mouhotii*). These turtles are well known as Asian "box turtles". Bramble (1974) pointed out the absence of substantial differences in the plastral hinge structure between *Cuora* and *Cistoclemmys*, and Sites *et al*. (1984), on the basis of allozymic analyses, suggested a closest affinity between these two genera. Based on these pieces of information, some authors regard *Cistoclemmys* as a junior synonym of *Cuora* (Ernst and Barbour, 1989; McCord and Iverson, 1991; Iverson, 1992; David, 1994; Fritz and Obst, 1997: Wu *et al*., 1999). Fritz and Obst (1997) further doubted the separation *Pyxidea* from *Cuora* sensu lato because of states of a few morphological characters in *Ci. serrata* that are intermediate between states of corresponding characters in *Cistoclemmys* and *Pyxidea*. However, this taxonomic account lost its base with the elucidation of actual origin of *Ci. serrata* as an intergeneric hybrid (Parham *et al*., 2001).

On the other hand, cladistic analyses of morphological characters suggested a distant location between *Cistoclemmys* and *Cuora* sensu stricto, and a closest affinity of the former with *Geoemyda* and *Pyxidea* (Fig. 1A: Hirayama

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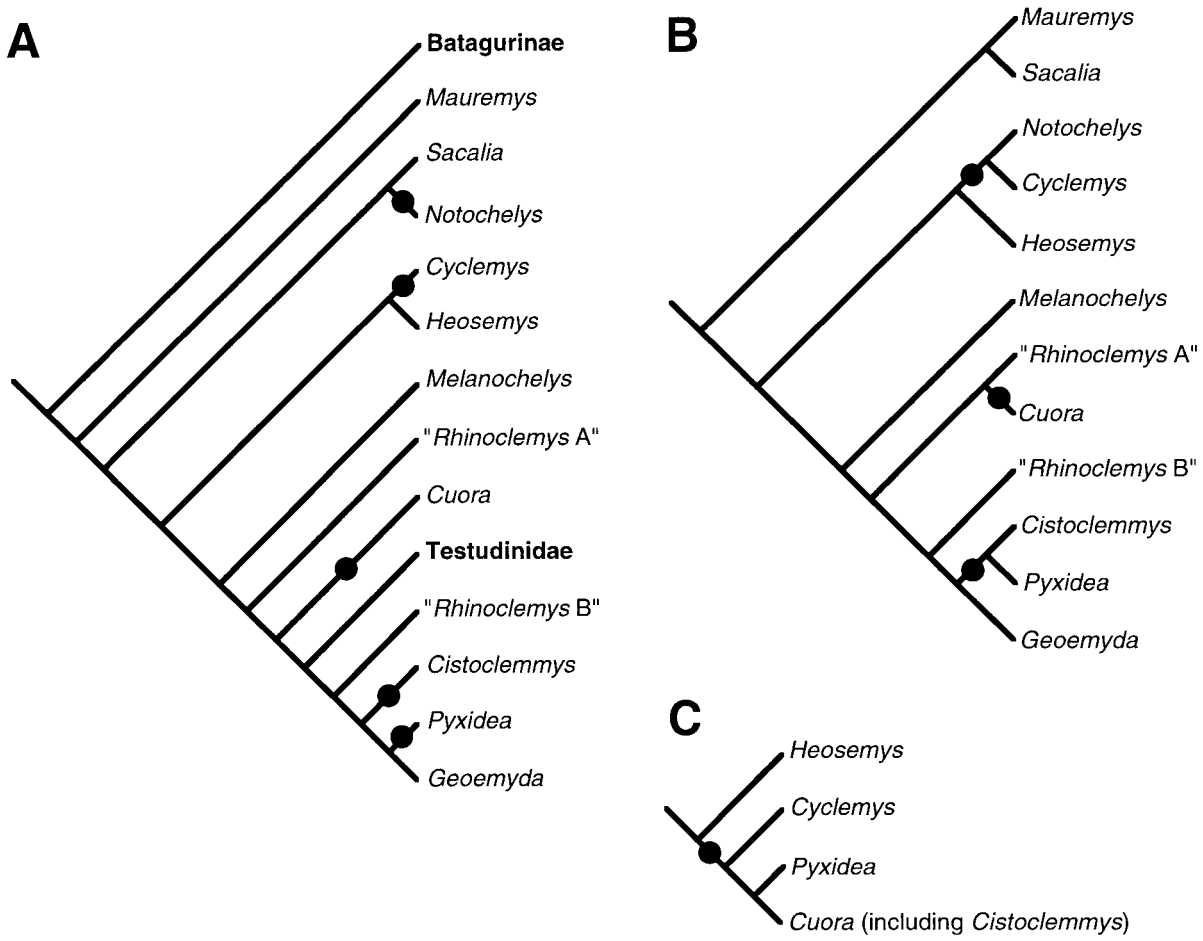


Fig. 1. Phylogenetic hypotheses of the Geoemydinae based on morphological characters. Circles denote positions of evolution of the plastral hinge. (A) Hirayama (1984: this view was also favored by Gaffney and Meylan [1988]). (B) Yasukawa *et al.* (2001). (C) Bramble (1974).

1984; Gaffney and Meylan, 1988), or solely with *Pyxidea* (Fig. 1B: Yasukawa *et al.*, 2001). Furthermore, Hirayama (1984) went so far as to suspect that the Testudinidae had derived from an ancestor exclusively common to some members of the Geoemydinae (i.e., some *Rhinoclemmys* species, *Geoemyda, Pyxidea* and *Cistoclemmys*: Fig. 1A). This view lead to serious controversy concerning the extent of application of the generic name *Cuora*, and several authors regarded *Cistoclemmys* as valid (Bour, 1980; Hirayama, 1984; Gaffney and Meylan, 1988; King and Bruke, 1989; Ota, 1991; Zhao and Adler, 1993; Yasukawa and Ota, 1999; Yasukawa *et al.*, 2001).

In these two decades, the genus *Cuora* sensu lato have also been receiving great attentions from a viewpoint of the species taxonomy, leading to descriptions, synonymizations and revalidations involving a large proportion of the species included (see McCord and Iverson [1991] for example). Moreover, the genus seems to be an ideal material for biogeographical studies, because members of *Cuora* sensu lato occur not only in the continental part but also over almost whole of the insular region of Southeast and East Asia (Iverson, 1992).

Recently molecular studies yielded advanced phylogenetic hypotheses for some badgered turtles (e.g., Shaffer *et al.*, 1997; Wu *et al.,* 1999; McCord *et al.*, 2000). However, none of these studies could appropriately address the phylogenetic, taxonomic and biogeographical problems of *Cuora* sensu lato, mainly due to the insufficiency in samples analyzed therein.

In the present study, we partially sequenced mitochondrial 12S and 16S ribosomal RNA (rRNA) genes for samples representing various testudinoid taxa including all but one species of *Cuora* sensu lato, and analyzed resultant data phylogenetically. Our purpose is to establish robust phylogenetic hypotheses for the Bataguridae with a particular emphasis on the interrelationships of *Cuora* and the presumptive relatives (see above). Also, we discuss implications of the resultant phylogeny on the taxonomy and biogeography of the Asian box turtles.

MATERIALS AND METHODS

Samples representing five of the six extant of *Cuora* sensu stricto, and the two species and one subspeices of *Cistoclemmys* (see McCord and Iverson, 1991; Yasukawa and Ota, 1999; Hilton-Taylor, 2000) were examined. We failed to examine specimens of *Cu. mccordi* due to the rarity of this recently described continental

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species (Ernst, 1988). Representatives of other genera of the Bataguridae, as well as those of the Emydidae, Testudinidae and other families, were also subjected to the analyses on the basis of newly obtained (Table 1, see Appendix for further details) and previously published data (Honda *et al*., 2002: Table 1). Of these, *Staurotypus triporcatus* of the Kinosternidae and *Pelodiscus sinensis* of the Trionychidae were regarded as outgroups, because these families are considered to be basal to the superfamily Testudinoidea (Gaffney and Meylan, 1988).

Extraction, amplification and sequencing procedures of DNA are described in detail elsewhere (Honda *et al*., 1999a,b). A part of mitochondrial 12S and 16S rRNA genes were amplified using the polymerase chain reaction (PCR) with primers L1091, H1478, L2606 and H3056 (Kocher *et al*., 1989; Hedges *et al*., 1993). Alignments for DNA sequences were determined based on maximum nucleotide similarity following Honda *et al.* (2000). The sequence data have been submitted to the DDBJ database, and their accession numbers are given in Table 1. Aligned data will be provided by the corresponding author on request.

The neighbor-joining (NJ) method (Saitou and Nei, 1987) was

Table 1. Batagurid and other species used for the present analyses. Data source are (a) Honda *et al*. (2002); (b) this study. See Appendix for further details.

Sample	Sub/family	McDowell (1964)	Bramble (1974)	Distribution
Callagur borneoensis[b]	Batagurinae	*Batagur* complex		S Thailand, Malaysia, W Indonesia
Chinemys reevesii[a]	Batagurinae	*Batagur* complex		Japan (Mainland), Korea, China (including Taiwan)
Hardella thurjii[b]	Batagurinae	*Hardella* complex		N India, Bangladesh, Pakistan
Orlitia borneensis[a]	Batagurinae	*Orlitia* complex		Malaysia, W Indonesia
Cistoclemmys f. flavomarginata[b]	Geoemydinae	*Geoemyda* complex	*Heosemy*s complex	S China (including Taiwan)
Cistoclemmys f. evelynae[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	Japan (S Ryukyu Is.)
Cistoclemmys galbinifrons[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	Vietnam, S China, Cambodia, Laos
Cuora amboinensis[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	South and Southeast Asia
Cuora aurocapitata[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	E China
Cuora pani[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	E and SE China
Cuora trifasciata[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	N Vietnam, Laos, S China
Cuora zhoui[b]	Geoemydinae	*Geoemyda* complex	*Heosemy* complex	SE China
Cyclemys sp.[a]	Geoemydinae	*Geoemyda* complex	*Heosemys* complex	S China, South and Southeast Asia
Geoemyda japonica[b]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	Japan (C Ryukyu Is.)
Heosemys grandis[b]	Geoemydinae	*Geoemyda* complex	*Heosemys* complex	Burma, Thailand, Cambodia, Vietnam, W Malaysia
Mauremys annamensis[a]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	C Vietnam
Mauremys caspica[a]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	SE Europe, SW former U.S.S.R., W Asia, Israel
Mauremys japonica[a]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	Japan (Mainland)
Mauremys mutica[a]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	N Vietnam, S China (including Taiwan), Japan (S Ryukyu Is.)
Notochelys platynota[b]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	S Burma, Thailand, Malaysia, W Indonesia, S Vietnam
Pyxidea mouhotii[b]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	S China, Vietnam, N Thailand, N Burma, E India
Sacalia bealei[a]	Geoemydinae	*Geoemyda* complex	*Geoemyda* complex	S China, N Vietnam
Trachemys scripta elegans[b]	Emydidae			S U.S.A., NE Mexico
Emys orbicularis[b]	Emydidae			Europe, W former U.S.S.R., W Asia, Israel, N Africa
Testudo horsfieldii[b]	Testudinidae			SW former U.S.S.R., W China, Iran, Afghanistan, Pakista
Geochelone carbonaria[b]	Testudinidae			S America
Staurotypus triporcatus[b]	Kinosternidae			C America
Pelodiscus sinensis[b]	Trionychidae			SE Russia, E China (including Taiwan), N Vietnam, Korea, Japan (Mainland)

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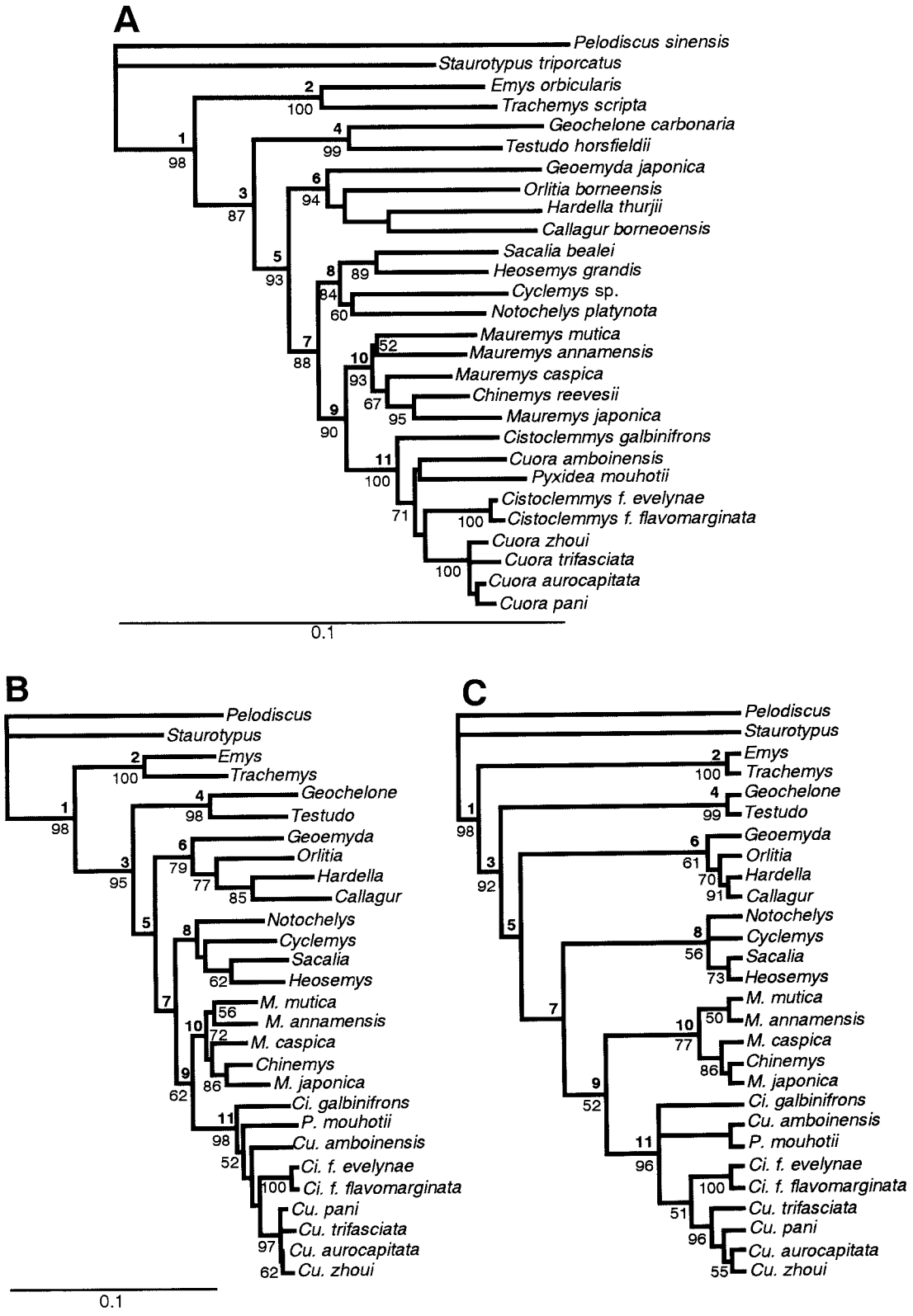


Fig. 2. (A) Neighbor-joining (NJ) dendrogram derived from distance matrix from 12S and 16S rRNA sequence data. Numbers beneath branches are BPs at least 50% of the 2,000 bootstrap replications. Nodes with bold numbers indicate relationships referred to in the text. Bar equals 0.1 unit of Kimura's two-parameter distance. (B) Maximum-likelihood (ML) dendrogram (ln likelihood=–6152.8). Branches without BP values were not supported in ≥50% of the 100 bootstrap replicates. Bar equals 0.1 unit. Bold numbers above branches are identical with those in NJ and MP dendrograms. (C) Maximum parsimony (MP) dendrogram using heuristic using heuristic option (strict consensus of four parsimonious trees, 894 steps, 208 bp informative under the condition of parsimony, consistency index=0.50, homoplasy index=0.50, retention index=0.54). Branches without BP values were not supported in ≥50% of the 2,000 bootstrap replicates.

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applied to infer relationships among taxa on the basis of a pairwise matrix of the distance from Kimura's (1980) two-parameter model. The maximum-likelihood (ML, with empirical base frequencies and equal rate substitution model) and maximum-parsimony analyses (MP, no bias between transition and transversion) were also conducted using heuristic search option. These analyses were performed by use of PAUP* 4.0b (Swofford, 1998) excluding gap sites, and confidences of branches were assessed by bootstrap resamplings (Felsenstein, 1985).

RESULTS

The 12S rRNA fragment consisted of 410 total sites, 168 of which were variable. For the 16S rRNA fragment, there were 472 total aligned sites, 178 of which were variable. Intergeneric nucleotide replacements within the Bataguridae varied from 16 bp (*Chinemys reevesii* vs. *Mauremys caspica*) to 95 bp (*Callagur borneoensis* vs. *Notochelys platynota*). Interspecific nucleotide replacements involved 34–35 bp, 2–47 bp, 20–26 bp and 2–47 bp in *Cistoclemmys*, *Cuora, Mauremys* and the *Cuora* group (assemblage of *Cuora, Cistoclemmys* and *Pyxidea*: see below), respectively. In continental *Cuora* (*Cu. aurocapitata, Cu. pani, Cu. trifasciata* and *Cu. zhoui*), nucleotide substitutions were observed in 2–11 bp, whereas three base-pairs were replaced between representatives of the two subspecies of *Ci. flavomarginata*.

The NJ dendrogram derived from the Kimura's (1980) distance matrix from aligned sequences is shown in Fig. 2A. The monophyly of the Testudinoidea was strongly supported (node 1: bootstrap proportion [BP] = 98%). The ingroup portion of this dendrogram was divided into two major clusters: the Emydidae (node 2: 100%), and an assemblage of the Testudinidae and the Bataguridae (node 3: 87%). The latter further split into two clusters, of which node 4 (99%) consisted of testudinid genera, whereas node 5 (93%) accommodated all the batagurid species examined. Within this last node, the *Callagur—Geoemyda—Hardella—Orlitia* assemblage (node 6: 94%) diverged first, followed by the *Cyclemys—Heosemys—Notochelys—Sacalia* (node 8: 84%) and the *Chinemys—Mauremys* assemblage (node 10: 93%) in order, leaving the *Cistoclemmys—Cuora—Pyxidea* assemblage as a monophyletic group (node 11: 100%).

Both the two subspecies of *Ci. flavomarginata* and members of Chinese *Cuora* (*Cu. aurocapitata, Cu. pani, Cu. trifasciata* and *Cu. zhoui*) constituted monophyletic groups with complete BP (100%) within the node 11. In contrast, monophyly was not supported for *Cistoclemmys* and *Cuora* sensu stricto due to the initial divergence of *Ci. galbinifrons* from the remaining node 11 components and an apparently distant affinity of *Cu. amboinensis* with the Chinese congeners, respectively.

Relationships resulting from ML (Fig. 2B) and MP analyses (Fig. 2C) were consistent with those expressed in the NJ dendrogram in terms of topology of the nodes 1–11, although they failed to support three of them (nodes 5, 7 and 8) with substantial BP values (i.e., >50%). Also, both analyses, while supporting monophyly of the two *Ci. flavomarginata* subspecies and of the Chinese *Cuora* with complete or nearly complete BPs, did not offer any support to the monophyly of *Cistoclemmys* and *Cuora* sensu stricto.

DISCUSSION

Bramble (1974), on the basis of the shell-closing mechanism, and morphology of the pelvic girdle and *Musclus testoscapularis*, assumed that *Cuora* sensu lato (as consisting of *Cuora* sensu stricto and *Cistoclemmys*), *Cyclemys* and *Pyxidea* diverged from a *Heosemys*-like ancestor (Fig. 1C). He also noted that there were no substantial differences in the plastral hinge mechanism between *Cu. amboinensis* and *Ci. flavomarginata*. This has been one of the major reasons to regard *Cistoclemmys* as a junior synonym of *Cuora* (see above). However, phylogenetic analyses of morphological data by Hirayama (1984) and Yasukawa *et al*. (2001) strongly suggested that *Cistoclemmys* constitutes a monophyletic group with *Geoemyda* and *Pyxidea*, and thus is distinct from *Cuora* (Fig. 1A, B).

Based on sequence data of mitochondrial cytochrome b gene, McCord *et al*. (2000) provided a phylogram showing the monophyly of *Cuora, Pyxidea* and three other genera exclusive of *Geoemyda*. This relationship contradicts with the hypothesis by Hirayama (1984) and Yasukawa *et al*. (2001) (see above). In the results of McCord *et al*. (2000), however, phylogenetic positions of the batagurid box turtles remained equivocal because McCord *et al*. combined those genera, including *Cuora* sensu lato, into one operational taxonomic unit, and did not illustrate any relationships among them. Our results thus provide for the first time a substantial support to the monophyly of *Cistoclemmys, Cuora* sensu stricto and *Pyxidea* (henceforth referred to as the *Cuora* group) (contra Bramble 1974; Hirayama, 1984; Yasukawa *et al*., 2001), and detailed relationships among their species.

Bramble (1974) postulated that in the Bataguridae the plastral hinge evolved in the common ancestor of *Cistoclemmys, Cyclemys, Cuora* and *Pyxidea* (Fig. 1C), and also in the lineage leading to *Notochelys* (not shown). However, results of the morphological analyses by Hirayama (1984) and Yasukawa *et al*. (2001), respectively, suggest that the hinge independently evolved five and three times in this family (Fig. 1A, B). Present results support none of these hypotheses, because they suggest that the plastral hinge emerged in the common ancestor of the *Cuora* group and supposedly in the common ancestor of *Cyclemys* and *Notochelys* (Fig. 2).

Phylogeny within the *Cuora* group resulting from our analyses is a great surprise in that it supports the monophyly for none of the genera *Cistoclemmys*, and *Cuora* in broad and strict senses so far proposed. *Cistoclemmys flavomarginata* is known to share a unique shape of the iliac blade exclusively with *Ci. galbinifrons* and this character state has thus been considered as a synapomorph of the genus (Yasukawa *et al*., 2001). However, relationships

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depicted in Fig. 2 suggest that this derived character state actually evolved independently in lineages leading to the two species.

Yasukawa and Ota (1999), on the basis of slight but somewhat consistent morphological differences, argued the recognition of the southern Ryukyu populations of *Ci. flavomarginata* as a valid subspecies, *Ci. f. evelynae*. Small, but yet substantial sequence divergence between the southern Ryukyu and Taiwanese samples of the species examined here seems to be congruent with this taxonomic account. It is probable that such morphological and genetic divergences between *Ci. flavomarginata* populations from southern Ryukyus and Taiwan correspond to the vicariance of these two areas through the insularization of the former, which supposedly took place during the middle-late Pleistocene (Ota, 1998).

With respect to the continental members of *Cuora* examined here, our analyses yielded robust supports not only for their monophyly, but also for their remarkably low sequence divergence which is almost comparable to the divergence between the two subspecies of *Ci. flavomarginata*. Species of *Cuora* distributed in the continent (China and southern Vietnam) are morphologically quite similar to each other and are diagnosed chiefly on the basis of distinct coloration (McCord and Iverson, 1991), whereas *Cu. amboinensis*, the only species of the genus occurring broadly in the Sunda Islands and the Philippines (Iverson, 1992), exhibits a greater number of morphological characters in unique states (e.g., McCord and Iverson, 1991; Yasukawa *et al*., 2001). Our results suggest that the ancestral lineage of *Cu. amboinensis* split relatively early in the *Cuora* group, and that speciations in the continental *Cuora* took place much later than that event, or speciations in other related genera, such as *Mauremys* (Fig. 2).

Our data, while strongly supporting the monophyly of the *Cuora* group as a whole, did not support any of the previous generic arrangements within the group. Unequivocal inclusion of *Pyxidea mouhotii* within the *Cuora* group and its closest affinity to *Cuora* sensu lato exclusive of *Ci. galbinifrons* are of particular interests, because the monotypic *Pyxidea* has never been synonymized with *Cuora* or *Cistoclemmys*, and its species was once assigned to *Cyclemys* (Wermuth and Mertens, 1961; Prichard, 1979; Xiangkui, 1994) or *Geoemyda* (McDowell, 1964). Considering this, as well as the non-monophyly of *Cistoclemmys* and *Cuora* sensu stricto, we suggest the application of the generic name *Cuora* to all species of the *Cuora* group by synonymizing *Cistoclemmys* and *Pyxidea* with this genus. This view definitely needs further verifications though.

McDowell (1964) suspected that the Bataguridae (as the subfamily Batagurinae of the family Testudinidae) was derived from a common ancestor with the Testudinidae as exclusive of the Emydidae (Emydinae of the Testudinidae). While most subsequent authors explicitly or implicitly assumed a closer affinity of the batagurid turtles with the emydids (e.g., Pritchard, 1979; Ernst and Barbour, 1989; Iverson, 1992), Hirayama (1984) progressed McDowell's (1964) view by arguing the sister relationship between an assemblage of some genera of the batagurid subfamily Geoemydinae and the Testudinidae on the basis of cladistic analyses of morphological characters (Fig. 1A). This view was approved by Gaffney and Meylan (1988).

Based on both molecular and morphological data, Shaffer *et al*. (1997) depicted the sister relationship between the Testudinidae and the Bataguridae. However, because their material included only two non-geoemydine batagurid genera, they failed to test Hirayama's (1984) hypothesis in a strict manner. Results of our analyses by incorporating data for most taxa crucial to those and related problems, while supporting McDowell's (1964) view for the sister relationships of the Bataguridae and the Testudinidae, negate Hirayama's (1984) view by supporting the monophyly of the Bataguridae. Also, our results do not support any of the subfamilial classifications and other generic grouping within the Bataguridae so far proposed on the morphological ground (Table 1). On the other hand, our results are largely concordant with those of more preliminary molecular studies dealing with turtles (Wu *et al*., 1999; McCord *et al*., 2000; Honda *et al*., 2002). We thus consider that in the testudinoid turtles morphological evolution often leads to an extensive homoplasy presumably under the operation of environmental selective forces.

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In Japan and Republic of China (Taiwan), handling of *Cistoclemmys flavomarginata* is strictly regulated by the respective governments. Tissue sample (tail tip) representing *C. f. evelynae* was obtained in the field on Ishigakijima under a permission from the Agency of Culture, Japan, through the courtesy of H. Nomura (Section of Culture, Education Committee, Prefectural Government of Okinawa). For the Taiwanese population (*C. f. flavomarginata*), tissue (tail tip) was sampled from an individual, captured in Feitsui Reservation of northern Taiwan and temporarily kept in captivity under a permission from Council of Agriculture, Republic of China (No. 900115339-A1. PDF), through the courtesy of T.-H. Chen (National Taiwan Ocean University, Keelung).

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APPENDIX

Catalogue numbers, sampling localities and DDBJ accession numbers of specimens. *Imported by a pet dealer (detailed localities unknown). **Obtained in the field (tail tip sample).

Callagur borneoensis: Herpetological Collection of the Department of Zoology, Kyoto University (KUZ) 47310*, AB090044, AB090072. *Chinemys reevesii:* Hyogo, Japan, KUZ36609, AB090026, AB090054. *Cistoclemmys f. flavomarginata*: Feitsui, Taiwan**, AB090035, AB090063. *Ci. f. evelynae*: Ishigakijima, Japan**, AB090034, AB090062. *Ci. galbinifrons*: KUZ36757*, AB090028, AB090056. *Cu. amboinensis*: KUZ36656*, AB090029, AB090057. *Cu. aurocapitata*: Ren Hirayama Personal Collection (RH) 915*, AB090030, AB090058. *Cu. pani*: RH901*, AB090031, AB090059. *Cu. trifasciata*: KUZ36709*, AB090032, AB090060. *Cu. zhoui*: RH868*, AB090033, AB090061. *Cyclemys* sp.: KUZ36654*, AB090027, AB090055. *Geoemyda japonica*: KUZ36655, AB090038, AB090066. *Hardella thurii*: KUZ36663*, AB090025, AB090053. *Heosemys grandis*: a living animal kept in Zoo Okinawa*, AB090039, AB090067. *Mauremys annamensis*: KUZ36700*, AB090041, AB090069. *M. caspica rivulata:* KUZ36680*, AB090043, AB090071. *M. japonica*: Kyoto, Japan, KUZ36599, AB090042, AB090070. *M. mutica kami*: Yonagunijima, Japan, AB090040, AB090068. *Notochelys platynota*: a living animal kept by M. Udagawa*, AB090037, AB090065**.** *Orlitia borneensis*: a living animal kept by Y. Yasukawa*, AB090024, AB090052. *Pyxidea mouhotii*: KUZ36723*, AB090036, AB090064. *Sacalia bealei:* a living animal kept in Zoo Okinawa*, AB090023, AB090051. *Trachemys scripta elegans*: an uncataloged tissue sample kept in KUZ*, AB090022, AB090050. *Emys orbicularis*: KUZ36652*, AB090021, AB090049. *Testudo horsfieldii*: a living animal kept by S. Nakai*, AB090020, AB090048. *Geochelone carbonaria:* KUZ47308*, AB090019, AB090047. *Staurotypus triporcatus:* KUZ36743*, AB090018, AB090046. *Pelodiscus sinensis*: an uncataloged tissue sample kept in KUZ*, AB090017, AB090045.

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