
The freshwater turtle genus *Mauremys* (Testudines, Geoemydidae) — a textbook example of an east–west disjunction or a taxonomic misconception?

DANA BARTH, DETLEF BERNHARD, GUIDO FRITZSCH & UWE FRITZ

Accepted: 9 July 2003

Barth, D. Bernhard, D. Fritsch, G. & Fritz, U. (2004): The freshwater turtle genus *Mauremys* — a textbook example of an east–west disjunction or a taxonomic misconception? — *Zoologica Scripta*, 33, 213–221.

We compare 1036 bp of the mitochondrial cytochrome *b* gene (*cyt b*) from all six *Mauremys* species with 16 other taxa, representing both currently recognized subfamilies of the Geoemydidae (Geoemydinae and Batagurinae) to contribute a comprehensive dataset towards resolving the conflicting *Mauremys* taxonomy and phylogeography. *Mauremys*, a representative of the Geoemydinae, is thought to be an example of a taxon with an east–west disjunction due to Pleistocene glacial extinction, with species occurring in the western Palearctic and species in the eastern Palearctic and Oriental regions. Our results contradict this traditional zoogeographical scheme and the current taxonomy of the Geoemydidae. *Mauremys* is paraphyletic with respect to two East Asian genera belonging to the Batagurinae: *Chinemys* and *Ocadia*. Therefore, *Mauremys*, as currently understood, clearly represents a taxonomic misconception. *Mauremys* + *Chinemys* + *Ocadia* contains four well supported clades, two of which — *M. japonica* + *Chinemys* + *Ocadia* and *M. annamensis* + *M. mutica* — are confined to eastern Asia. The other two — *M. caspica* + *M. rivulata* and *M. leprosa* — occur in the western Palearctic. *Mauremys leprosa* may represent an ancient lineage which differentiated before the split between the other western and eastern species occurred. The patchy distribution of the four clades is likely the result of several ancient radiations rather than of a Pleistocene extinction. The sister-group of *Mauremys* + *Chinemys* + *Ocadia* is *Cuora*, a morphologically highly specialized genus with a complicated shell hinging mechanism.

Dana Barth & Detlef Bernhard, University of Leipzig, Institute of Zoology, Molecular Evolution & Animal Systematics, Talstr. 33, 04103 Leipzig, Germany

Guido Fritsch, University of Leipzig, Interdisciplinary Centre for Bioinformatics (IZBI), Kreuzstr. 7b, 04103 Leipzig, Germany

Uwe Fritz, Zoological Museum, Natural History State Collections Dresden, A. B. Meyer Building, Königsbrücker Landstr. 159, 01109 Dresden, Germany. E-mail: uwe.fritz@snsd.smwk.sachsen.de

Introduction

The freshwater turtle genus *Mauremys* belongs to the Geoemydidae, a family consisting of approximately 60 species in 25 genera. Geoemydid turtles are mainly distributed in eastern Asia. Only three *Mauremys* species occur in the western Palearctic, and one genus (*Rhinoclemmys*) is distributed within central and northern South America (Ernst *et al.* 2000; Fritz 2001). Until recently, the family Geoemydidae Theobald, 1868 was better known under its junior synonym Bataguridae Gray, 1869. However, Bour & Dubois (1986) demonstrated that Geoemydidae is the nomenclaturally valid name.

Mauremys has a patchy distribution, including parts of the western Palearctic region and, separated by a huge disjunction, parts of the Oriental and eastern Palearctic regions

(Fig. 1). It is currently thought to consist of six species, three in the west and three in the east (Fritz 2001). The three western Palearctic species are *M. leprosa*, *M. rivulata* and *M. caspica*. *M. leprosa* inhabits western North Africa (Morocco to western Libya) and the Iberian Peninsula (Keller & Busack 2001). *M. rivulata* is distributed, in Europe, along the Adriatic coast southwards from central Dalmatia over Albania and Greece to Bulgaria; it is also found in many islands in the Ionic and Aegean Seas and in Crete and Cyprus. In Asia Minor, it is confined to the coastal regions of western and southern Turkey and stretches southwards along the Levantine coast to Israel (Fritz & Wischuf 1997; Wischuf & Busack 2001). *M. caspica* occurs in central Anatolia, the eastern Caucasus and Transcaucasus, Syria, Iraq, Iran, and western Turkmenistan.

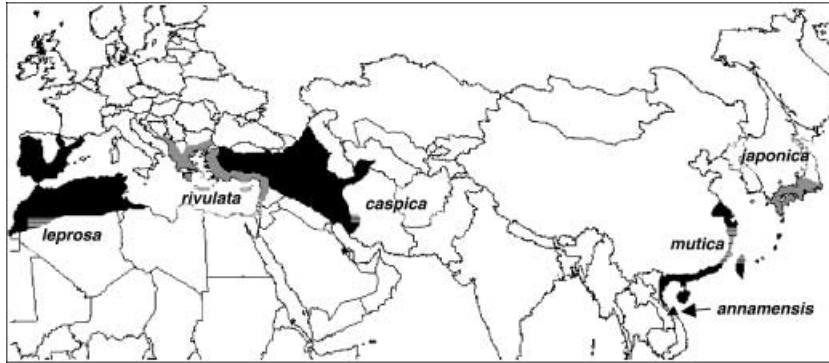


Fig. 1 Distribution of all currently accepted *Mauremys* species based on Iverson (1992), Fritz & Wischuf (1997), Keller & Busack (2001) and Wischuf & Busack (2001).

Its southernmost outposts are in the southern Persian Gulf, and include Bahrein and Saudi Arabia (Fritz & Wischuf 1997; Wischuf & Fritz 2001). The eastern species are *M. mutica*, *M. japonica* and *M. annamensis*. *M. mutica* occurs in Vietnam, southern China and the Japanese Ryukyu Islands. *M. japonica* is confined to the main islands of Japan, while *M. annamensis*, which was transferred from the monotypic genus *Annamemys* to *Mauremys* by Iverson & McCord (1994), is restricted to Vietnam (Iverson & McCord 1994; McCord 1997). Two further taxa described as new *Mauremys* species in the 1990s were demonstrated to be of hybrid origin. *M. iversoni* Pritchard & McCord, 1991 originated from hybridization of *M. mutica* with *Cuora trifasciata* (Parham *et al.* 2001; Wink *et al.* 2001), *M. pritchardi* McCord, 1997 from hybridization of *M. mutica* with *Chinemys reevesii* (Wink *et al.* 2001).

Other taxa which share similarly disjunct distributions are *Cyanopica cyanus* (Aves; Sedlag 1995; Fok *et al.* 2002), *Misgurnus fossilis* (Osteichthyes; Lattin 1967; Sedlag 1995), and among the class Amphibia the *Rana 'esculenta'–nigromaculata* complex, the genus *Bombina* and the Old World representatives of *Hyla* (Borkin 1984, 1986; Zug 1993). The phenomenon is generally thought to be the result of glacial extinctions during the Pleistocene (Lattin 1967; Sedlag 1995; Lapparent de Broin 2001).

Virtually all previous studies dealing with the systematics of *Mauremys* have been based on osteology and general morphology (McDowell 1964; Busack & Ernst 1980; Hirayama *et al.* 1985; Pritchard & McCord 1991; Iverson & McCord 1994; Yasukawa *et al.* 1996, 2001; Fritz & Wischuf 1997; McCord 1997). The monophyly of the genus was, until recently, never questioned and the East Asiatic genus *Sacalia* was generally accepted as its sister-group (McDowell 1964; Hirayama 1985; Yasukawa *et al.* 2001). The first molecular studies, although based on only a limited dataset, could not confirm a close relationship between *Mauremys* and *Sacalia* (Wu *et al.* 1999; McCord *et al.* 2000; Honda *et al.* 2002a), while a close relationship between *M. japonica* and the East Asiatic genus *Chinemys* was demonstrated, suggesting a paraphyletic *Mauremys* (Honda *et al.* 2002a).

Here we compare 1036 bp of the mitochondrial cytochrome *b* gene (*cyt b*) from all six *Mauremys* species with 16 other taxa, representing both currently recognized subfamilies of the Geoemydidae (Geoemydinae and Batagurinae; Gaffney & Meylan 1988) with the aim of providing a comprehensive dataset which may help resolve the conflicts between the taxonomy and phylogeography of *Mauremys*.

Materials and methods

Sampling

Tissue or blood samples were obtained from 25 specimens belonging to 22 species (Table 1). These samples represent all currently recognized species of the genera *Mauremys*, *Chinemys* and *Sacalia*, and representative species of other major groups of the Geoemydidae. Tissue samples from thigh muscles were obtained by dissecting freshly killed animals. All specimens were identified by two specialists. Complete alcohol-preserved specimens are deposited in the herpetological collection of the Zoological Museum Dresden (= Museum für Tierkunde Dresden, MTD) under the catalogue numbers listed in Table 1. Blood samples were acquired by coccygeal vein puncture as described in Haskell & Pokras (1994). Blood and tissue samples were stored at -70°C in ethanol or EDTA buffer (Arctander 1988).

DNA Extraction, PCR amplification and sequencing

Total genomic DNA was extracted from thigh muscle tissues following the protocol of Gustincich *et al.* (1991), while DNA from blood samples was isolated using the QIAamp Blood Mini Kit (Qiagen).

Slightly modified versions of the primers mt-A (Lenk & Wink 1997) and H15909 (Lenk *et al.* 1999) were used to amplify a fragment of approximately 1080 bp containing 1036 bp of *cyt b* and part of the tRNA threonine (Table 2). PCR conditions were as follows: 5 min at 95°C , then 40 cycles of 1 min at 95°C , 1 min at 50°C , 2 min at 72°C , and a single extension step of 10 min at 72°C .

Sequencing reactions were performed with the 7-deaza-dGTP sequencing kit (Amersham Pharmacia) and separated

Table 1 Specimens examined in this study. (MTD, Museum für Tierkunde Dresden).

Taxon	Locality	Voucher number/Origin	EMBL acc. no.
SUBFAMILY GEOEMYDINAE			
<i>Mauremys annamensis</i> (Siebenrock, 1903)	Vietnam	MTD live collection	AJ564456
<i>M. caspica caspica</i> (Gmelin, 1774)	Turkey: Birecik	MTD 42628	AJ564453
<i>M. caspica siebenrocki</i> Wischuf & Fritz, 1997	Bahrain	live collection, Breeding Centre for Endangered Arabian Wildlife, Sharja, UAE	AJ564454
<i>M. japonica</i> (Temminck & Schlegel, 1835)	Unknown	MTD 42498	AJ564458
<i>M. leprosa</i> (Schweigger, 1812)	Spain: Doñana Biological Reserve	wild specimen	AJ564457
<i>M. mutica mutica</i> (Cantor, 1842)	Unknown	MTD 41973	AJ564459
<i>M. mutica</i> cf. <i>kami</i> Yasukawa, Ota & Iverson, 1996	Unknown	MTD live collection	AJ564460
<i>M. rivulata</i> (Valenciennes, 1833)	Turkey: Izmir	MTD 41974	AJ564455
<i>Sacalia bealei</i> (Gray, 1831)	Unknown	MTD 41583	AJ519501
<i>S. quadriocellata</i> (Siebenrock, 1903)	China: Canton (market)	MTD 42442	AJ564465
<i>Notochelys platynota</i> (Gray, 1834)	Unknown	MTD 41947	AJ564462
<i>Leucocephalon yuwonoi</i> (McCord, Iverson & Boeadi, 1995)	Indonesia: Sulawesi	MTD 42572	AJ564450
<i>Melanochelys trijuga edeniana</i> (Theobald, 1876)	Myanmar: Kachin province	MTD 41813	AJ564461
<i>Cuora amboinensis amboinensis</i> (Daudin, 1801)	Indonesia: Sulawesi	MTD 41811	AJ564447
<i>C. galbinifrons galbinifrons</i> Bourret, 1939	Northern Vietnam	MTD 44118	AJ564448
<i>Geoemyda spengleri</i> (Gmelin, 1789)	Unknown	MTD 41930	AJ564449
SUBFAMILY BATAGURINAE			
<i>Kachuga dhongoka</i> (Gray, 1834)	Unknown	MTD 42577	AJ564452
<i>Ocadia sinensis</i> (Gray, 1834)	China	MTD 42594	AJ564463
<i>Hieremys annandalei</i> (Boulenger, 1903)	Cambodia: Phnom Penh	MTD 42517	AJ564451
<i>Chinemys megaloccephala</i> Fang, 1934	Unknown	MTD 41809	AJ519498
<i>C. megaloccephala</i> Fang, 1934	China	MTD 41904	AJ519499
<i>C. nigricans</i> (Gray, 1834)	China	MTD 42864	AJ519500
<i>C. reevesii</i> (Gray, 1831)	China	MTD 41905	AJ519497
<i>Malayemys subtrijuga</i> (Schlegel & Müller, 1844)	Unknown	MTD 43718	AJ519502
<i>Orlitia borneensis</i> Gray, 1873	Unknown	MTD 42499	AJ564464

on an automated LI-COR DNA sequencer. Both strands were sequenced using mt-A and H15909 (see above) and the internal primers mt-c2, mt-e, mt-E and TestudRi3. These primers are modified versions of the primers used by Wink (1995) and Lenk *et al.* (1999), with the exception of the newly designed TestudRi3 (Table 2).

Phylogenetic analyses

MEGA v. 2.1 (Kumar *et al.* 2001) was used to estimate genetic distances and to calculate sequence statistics. Alignment was carried out with ClustalX v. 1.8 (Thompson *et al.* 1997) with default parameters.

Maximum likelihood (ML) trees were calculated with PAUP* 4.0 b10 (Swofford 2002) and TREE-PUZZLE v. 5.0 (Schmidt *et al.* 2002). To find the most appropriate model of DNA substitution we carried out a hierarchical likelihood ratio test with Modeltest v. 3.06 (Posada & Crandall 1998). A model which combined GTR (Rodriguez *et al.* 1990), gamma (G; shape parameter = 1.0223) of site-specific rate heterogeneity (Yang 1994) and invariable sites (I = 0.4648) was singled out as the best for the whole dataset. For the pruned dataset

Table 2 Primers used in this study. Numbers refer to positions of the 3' ends of the primers in the mitochondrial genome of *Chrysemys picta* (Mindell *et al.* 1999).

Primer	Sequence	Position
mt-A	5'-CAACATCTCAGCATGATGAACTTCG-3'	L 14501
mt-c2	5'-GAGGACAAATATCATTCTGAGG-3'	L 14826
mt-e	5'-AAACCAGAATGATACTTCTATTTCG-3'	L 15231
H15909	5'-CAGTTTTTGGTTTACAAGACCAATG-3'	H 15569
mt-E	5'-GCAAATAGGAAGATCATTCTGG-3'	H 15209
TestudRi3	5'-AGTAGGTTGGTATGACAGTGGC-3'	H 14828

(see Results) HKY85 (Hasegawa *et al.* 1985) plus G (shape parameter = 0.2248) proved to be the best. These results were used in the ML calculations. In PAUP*, the heuristic search method was invoked with 100 random stepwise additions and the TBR branch-swapping algorithm. Bayesian phylogenetic analysis was carried out with MrBayes (Huelsenbeck & Ronquist 2001), which was used to run 1 000 000 generations, with a sampling frequency of 10 generations. From the 100 000 trees found, the first 5000 were discarded.

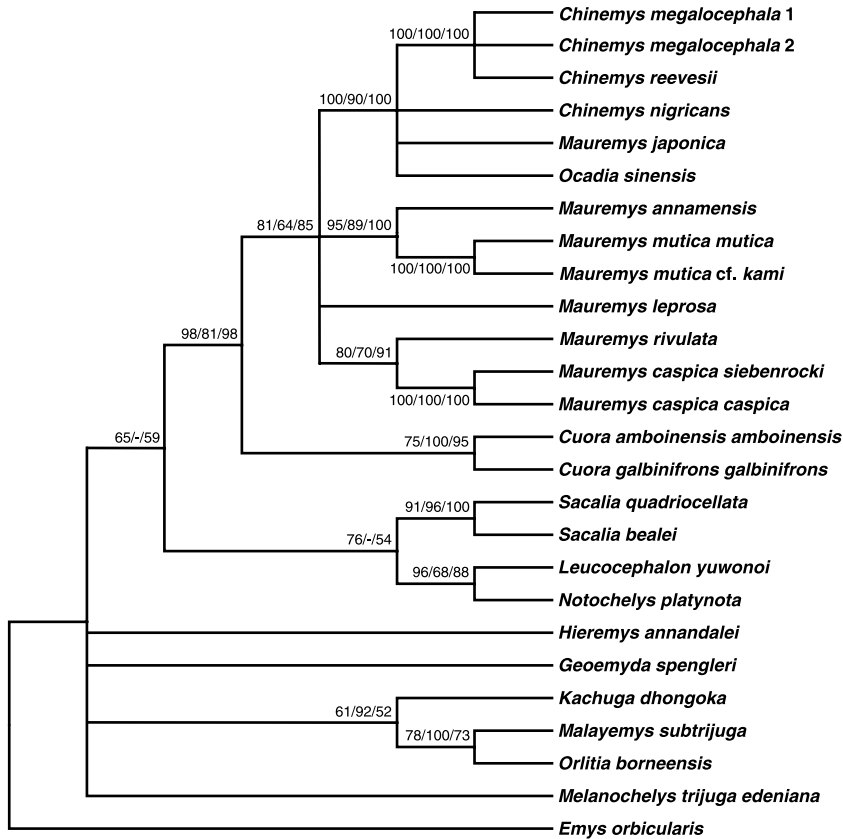


Fig. 2 Maximum parsimony (MP) tree of the Geoemydidae inferred from cytochrome *b* sequences. *Emys orbicularis* was chosen as outgroup. The first numbers at the nodes represent bootstrap values out of 1000 trees in MP analysis. Nodes supported by values below 50% are shown as multifurcations. The second numbers give bootstrap values (100 bootstrap resamplings) using the maximum likelihood method (GTR + G + I) in PAUP, while the third are bootstrap values for 1000 bootstrap resamplings from the neighbour-joining analysis using the same model of substitution.

Neighbour-joining (NJ) trees (Saitou & Nei 1987) were also constructed with PAUP*. We chose models and parameters as selected by Modeltest. Additionally, the models of Kimura (1980) and Tamura & Nei (1993) were used, which yielded the same tree topologies and nearly identical bootstrap values. Maximum parsimony (MP) analyses were performed with PAUP* using the heuristic search method with 10 random stepwise additions and the TBR branch swapping option.

Bootstrap analyses (Felsenstein 1985) were used to examine the robustness of the resulting bifurcations within the trees. MP and NJ trees were tested with 1000 replicates. Because of the enormous computational time only 100 bootstrap resamplings were carried out in the ML analyses. In TREE-PUZZLE quartet puzzling support values were calculated for each branch, which are comparable to bootstrap values (Strimmer & Haeseler 1996).

The European pond turtle *Emys orbicularis* (Accession no. AF258868; Feldman & Parham 2002) from the closely related family Emydidae (Gaffney & Meylan 1988; Shaffer *et al.* 1997) was used to root the trees of the complete dataset.

Results

For phylogenetic analyses we sequenced 1036 bp of cyt *b* from all currently accepted species of *Mauremys* and from

representatives of 12 other genera of the family Geoemydidae. Within the alignment, 462 positions were variable and 332 parsimony informative. The overall Ti/Tv ratio was 4.7, ranging from 1.9 to 12.5 for each pairwise species comparison. Uncorrected pairwise sequence divergence ranged from 0.5% between subspecies of *M. mutica* and 1.1% between subspecies of *M. caspica* to 20.2% between the outgroup *Emys orbicularis* and *Malayemys subtrijuga*. No differences were detected between the sequences of *Chinemys megalocephala* and *C. reevesii*.

In all tree reconstruction methods used, *Mauremys*, *Chinemys* and *Ocadia* represent a monophylum (Fig. 2). However, within this clade, *Mauremys* is paraphyletic. The six species cluster into different groups; *M. japonica* is embedded in *Chinemys* and *Ocadia*. Within *M. japonica* + *Chinemys* + *Ocadia* the branching pattern varies between the analysis methods. The species from the Oriental region, *M. annamensis* and *M. mutica*, cluster together, as do *M. caspica* and *M. rivulata* from the western Palearctic. The position of *M. leprosa* differs according to the tree building method used. This species from the Iberian Peninsula and northern Africa represents an unresolved lineage. In MP analysis, *M. leprosa* and the other groups branch off in a multifurcation (Fig. 2). In the ML analysis (GTR + G + I; not shown), it is related to *M. caspica* and *M. rivulata*. In contrast, in the NJ analysis (same model and parameters; not

shown) it seems to be the most basal taxon within the whole *Mauremys* + *Chinemys* + *Ocadia* group. In all trees obtained the basal branches differ between the methods used and their bootstrap values are generally low. However, all tree building methods clearly support a sister-group relationship between the *Mauremys* + *Chinemys* + *Ocadia* clade and the genus *Cuora*. Within the *Mauremys* + *Chinemys* + *Ocadia* group as well as within the *M. japonica* + *Chinemys* + *Ocadia* subgroup, differences between methods occur, leading to unresolved nodes. As rising numbers of distantly related taxa in an analysis can increase levels of homoplasy (e.g. Lecointre *et al.* 1994; Philippe *et al.* 2000), we excluded all distantly related taxa to get a more detailed picture about phylogeny within *Mauremys* and the closely related genera *Chinemys* and *Ocadia*.

The new dataset comprised 1036 aligned positions from the species of *Mauremys*, *Chinemys* and *Ocadia*, with two *Cuora* species as outgroups. Of these positions, 269 were variable and 160 parsimony informative. The pairwise sequence divergence ranged up to c. 10% between the outgroup and ingroup species. Ti/Tv ratios increased as the genetic divergence among taxa decreased, and were thus higher among these species with an overall ratio of 5.9.

The phylogenetic analyses with this pruned dataset yielded trees with higher bootstrap or quartet puzzling support values than the former analyses (Fig. 2). Again, in none of the resulting trees did *Mauremys* form a monophylum and its six species clustered into four well supported groups (Fig. 3). *Mauremys japonica* was consistently associated with *Chinemys* and *Ocadia*. While MP and ML revealed a sister-group relationship between *Ocadia sinensis* and *M. japonica* as well as a monophyletic *Chinemys* (Fig. 3A), the other methods could not resolve this branching pattern sufficiently (Fig. 3B). Two other groups were formed by *M. annamensis* and *M. mutica* from the Oriental region, and *M. caspica* and *M. rivulata* from the western Palearctic. However, the branching pattern between these groups varied and could not be resolved unambiguously. MP and ML supported a sister-group relationship between *M. annamensis* + *M. mutica* and *M. caspica* + *M. rivulata* (Fig. 3A), although bootstrap support values for this scenario were low. Moreover, ML based on quartet puzzling, NJ and Bayesian analyses put *M. japonica* + *Chinemys* + *Ocadia*, *M. annamensis* + *M. mutica* and *M. caspica* + *M. rivulata* in a multifurcation (Fig. 3B). Again, *M. leprosa* represented its own clade, clearly separate from the other species of the western Palearctic. However, in the pruned analyses the basal branching of *M. leprosa* in the entire *Mauremys* + *Chinemys* + *Ocadia* group was stable and supported by high bootstrap or quartet puzzling support values.

Discussion

Our results contradict the current systematics within the Geoemydidae. According to Gaffney & Meylan (1988), the

Geoemydidae consists of two subfamilies, the Geoemydinae (e.g. *Mauremys*, *Cuora*) and the Batagurinae (e.g. *Chinemys*, *Ocadia*). However, we found support for a clade containing taxa from both currently recognized subfamilies (Figs 2, 3). The monophyly of *Mauremys* is generally accepted by most morphological studies (McDowell 1964; Busack & Ernst 1980; Hirayama 1985; Pritchard & McCord 1991; Iverson & McCord 1994; Yasukawa *et al.* 1996, 2001; Fritz & Wischuf 1997; McCord 1997) largely based on osteological investigations by McDowell (1964). First sequence comparisons of 16S and 12S rRNA data did not support a monophyly of *Mauremys* (Honda *et al.* 2002a) in that a closer affinity between *M. japonica* and *Chinemys reevesii* was detected rather than between *M. japonica* and other *Mauremys* species (*M. caspica*, *M. leprosa* and other *Chinemys* species were not studied).

All of our phylogenetic analyses based on the *cyt b* sequences suggest that *Mauremys* is paraphyletic, whereas *Mauremys*, *Chinemys* and *Ocadia* together form a monophyletic group. Within this clade, four distinct lineages are detected:

- 1 *M. japonica* + *Chinemys* + *Ocadia*
- 2 *M. annamensis* + *M. mutica*
- 3 *M. caspica* + *M. rivulata*
- 4 *M. leprosa*.

However, the sister-group relationships between these lineages could not be resolved unambiguously, even with the pruned dataset. Within *M. japonica* + *Chinemys* + *Ocadia*, MP and ML (Fig. 3A) indicated a closer relationship between *M. japonica* and *O. sinensis* than to the *Chinemys* species, although support values were low. The ribosomal sequence data of Honda *et al.* (2002a) do not clarify the phylogenetic relationships within that clade as *Ocadia*, *Chinemys megalcephala* and *C. nigricans* were not studied. According to our data, the *cyt b* sequences of *C. reevesii* and *C. megalcephala* are identical, suggesting that they either belong to the same species or that one of them is of hybrid origin. This problem is addressed elsewhere in detail (Barth *et al.* 2003).

Our methods confirm the close phylogenetic relationship between *M. annamensis* and *M. mutica* from eastern Asia as anticipated by morphological investigations (Iverson & McCord 1994; Yasukawa *et al.* 2001). Our results are also in line with those of Honda *et al.* (2002b). Further, our data support a close relationship of the western Palearctic species *M. caspica* and *M. rivulata*. This is also reflected by a similar gross morphology (Busack & Ernst 1980; Fritz & Wischuf 1997). Based on their geographical distribution (Fig. 1) and on phenetic characters, these species were previously thought to be closely related to *M. leprosa* (Loveridge & Williams 1957; Iverson & McCord 1994; McCord 1997; Fritz 2001; Lapparent de Broin 2001). Some authors even regarded these three taxa as subspecies (Loveridge & Williams 1957; Wermuth & Mertens 1961, 1977). On the other hand, earlier studies based on enzyme electrophoresis (Merkle 1975)

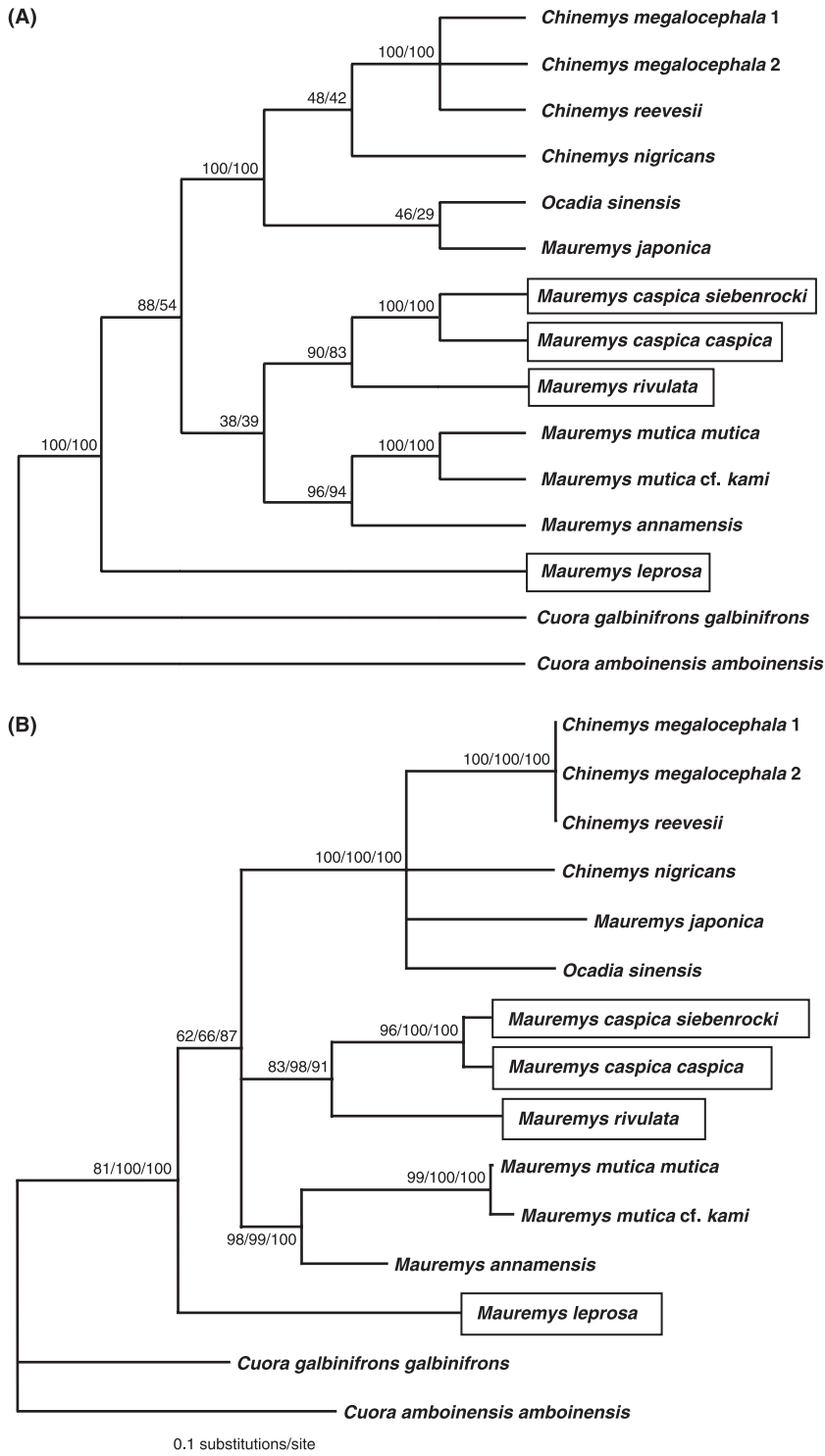


Fig. 3 A, B. Phylogenetic trees of the genera *Mauremys*, *Chinemys* and *Ocadia* using the two *Cuora* species as outgroups. Names of western Palearctic taxa in boxes, of East Asiatic taxa without boxes. —A. MP tree. The first numbers give the bootstrap values out of 1000 trees. The same topology was inferred with the maximum likelihood (ML) method (HKY85 + G) in PAUP; the second are bootstrap values for 100 bootstrap resamplings. —B. ML tree obtained with TREE-PUZZLE (HKY85 + G) with 10 000 steps and showing ML branch lengths. Scale bar = 0.1 nucleotide substitutions per site. The first numbers represent quartet puzzling support values. Nodes supported by values below 50% are given as multifurcations. Identical tree topologies were obtained with Bayesian and neighbour-joining analyses using the same model. The second numbers at the nodes represent the percentage of trees containing that grouping with Bayesian phylogenetic analysis, while the third numbers give bootstrap values for 1000 bootstrap resamplings of the dataset from the neighbour-joining analysis.

and morphometry (Busack & Ernst 1980) revealed a clear differentiation between *M. leprosa* and the other two taxa. Our analyses present additional evidence that *M. leprosa* is clearly distinct from *M. caspica* and *M. rivulata*. In all analyses of the pruned dataset, *M. leprosa* appears as the most basal taxon of *Mauremys* + *Chinemys* + *Ocadia* (Fig. 3). This suggests that *M. leprosa* might represent an ancient lineage, which branched off before the differentiation between *M. japonica* + *Chinemys* + *Ocadia*, *M. annamensis* + *M. mutica* and *M. caspica* + *M. rivulata* took place.

According to our data, the similarity of the species lumped together in *Mauremys* seems to be based on homoplastic morphological characters. *Mauremys* as defined hitherto is composed of four distinct clades which together form a monophylum. Two contain exclusively East Asiatic species; one includes two other genera (*Chinemys*, *Ocadia*). The other two consist of western Palearctic species. One of the western Palearctic clades, *M. leprosa*, appears to be the sister-taxon of all the other groups. Therefore, *Mauremys*, as currently understood, clearly represents a taxonomic misconception.

For many chelonians, a molecular clock of 0.4% sequence divergence per Myr is accepted for *cyt b* as well as for the complete mitochondrial genome (Avice *et al.* 1992; Bowen *et al.* 1993; Caccone *et al.* 1999; Lenk *et al.* 1999). If this rate is applied to our data, the four clades would have separated 18–23 Mya, i.e. in the Late Oligocene or Early Miocene. To find out whether our mtDNA sequences are indeed evolving in a clock-like fashion, we performed the Likelihood Ratio Test as implemented in TREE-PUZZLE. The results indicate that the *Mauremys*, *Chinemys* and *Ocadia* sequences did not evolve in this way. This questions the supposition that the mitochondrial genome in chelonians generally evolves in a clock-like fashion. Nevertheless, as the eastern and western species of *Mauremys* are not very closely related, their patchy distribution is likely to be the result of several ancient radiation events rather than of a recent (Pleistocene) extinction.

Morphological data suggest that *Mauremys* and *Sacalia* are closely related (McDowell 1964; Hirayama 1985; Yasukawa *et al.* 2001). Wermuth & Mertens (1977) even regard both as congeneric. Previous biochemical and molecular studies (Sites *et al.* 1984; Wu *et al.* 1999; McCord *et al.* 2000; Honda *et al.* 2002a, b) have not confirmed a sister-group relationship of *Sacalia* and *Mauremys*. This is in accordance with our results. Instead, we have detected a well supported sister-group relationship between *Cuora* and the complex containing *Mauremys*, *Ocadia* and *Chinemys*. McDowell (1964) pointed out that the skulls of *Cuora* and *Mauremys* are similar. *Cuora* is a highly specialized genus with terrestrial and aquatic species, known as ‘Asiatic box turtles’. All are characterized by a complicated shell morphology with a plastral hinge that allows entire shell closure (Bramble 1974; Ernst *et al.* 2000). In contrast, *Mauremys*, *Ocadia* and *Chinemys* represent

characteristic aquatic terrapins with a rigid plastron. Due to this obvious difference, the possibility of a close relationship was never investigated. The close relationship between *Cuora* and *Chinemys* + *Mauremys* + *Ocadia* could explain the frequently reported hybrids between *Cuora* and the other genera. These hybrids are vital and (partly) even fertile (Yasukawa *et al.* 1992; Shi & Parham 2001; Wink *et al.* 2001; Parham *et al.* 2001; Fritz & Mendau 2002; Galgon & Fritz 2002).

In contrast to our results, the 12S rRNA data of Wu *et al.* (1999) do not corroborate a sister-group relationship between *Cuora* and *Chinemys* + *Mauremys* + *Ocadia*. However, their cladogram is based on a quite short sequence of 400 bp and for the crucial branches no support values are provided. Therefore, their finding might be due to a hard polytomy or phylogenetic noise. This hypothesis is supported by the fact that Honda *et al.* (2002b) found a sister-group relationship between *Mauremys* + *Chinemys* and *Cuora* by using both 12S and 16S rRNA data.

Our data clearly recommend substantial taxonomic changes and even question the geoemydid subfamilies as recognized by Gaffney & Meylan (1988). However, as *Mauremys*, *Chinemys* and *Ocadia* form a monophyletic group, there are two methods of resolving this situation on the generic level: (1) lump all species into an expanded genus *Mauremys*, or (2) split *Mauremys* into four genera, reflecting the four clades contained in *Mauremys s.l.* + *Chinemys* + *Ocadia*. To decide which taxonomic arrangement is more appropriate, additional evidence from other geoemydid genera should be awaited. For the time being, it may be noted that *Ocadia* Gray, 1870 is the oldest available name for the clade containing ‘*M.*’ *japonica*, all *Chinemys* species, and *O. sinensis*. For the clade containing ‘*M.*’ *annamensis* and ‘*M.*’ *mutica*, *Cathaiemys* Lindholm, 1931 is available, and *Emmenia* Gray, 1870 for ‘*M.*’ *caspica* and ‘*M.*’ *rivulata*. *Mauremys* Gray, 1869 would have to be restricted to *M. leprosa* (for synonymies see Wermuth & Mertens 1977).

To get a more detailed picture of the phylogeny within the Geoemydidae, all genera have to be examined. However, our results demonstrate that *cyt b* alone cannot resolve the phylogenetic relationships. That applies in particular to the basal branches of the family (Fig. 2). As molecular and current morphological datasets are obviously conflicting, the future challenge will be not only to sequence additional genes but to identify and eliminate homoplastic morphological characters from phylogenetic analyses, leading to an integrated approach for a better understanding of the taxonomy and evolution of this family of archaic reptiles.

Acknowledgements

We wish to thank Martin Schlegel and two anonymous reviewers for constructive comments on the manuscript. Paul Vercammen provided blood samples of turtles in the care of

the Breeding Centre for Endangered Arabian Wildlife, Sharjah, UAE and Claudia Keller from specimens living in the Doñana Reserve, Spain.

References

- Arctander, P. (1988). Comparative studies of avian DNA by restriction fragment polymorphism analysis. *Journal of Ornithology*, 129, 205–216.
- Avise, J. C., Bowen, B. W., Lamb, T., Meylan, A. B. & Bermingham, E. (1992). Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. *Molecular Biology and Evolution*, 9, 457–473.
- Barth, D., Bernhard, D., Guicking, D., Stöck, M. & Fritz, U. (2003). Is *Chinemys megaloccephala* Fang, 1934 a valid species? New insights based on mitochondrial DNA sequence data. *Salamandra*, 38, 233–244.
- Borkin, L. J. (1984). The European–Far Eastern disjunctions in Amphibian distribution: a new analysis of the problem. *Proceedings of the Zoological Institute of the USSR Academy of Sciences, Leningrad*, 124, 55–88 [in Russian].
- Borkin, L. J. (1986). Pleistocene glaciations and western-eastern paleartic disjunctions in Amphibian distribution. Studies in Herpetology. *Proceedings of the European Herpetological Meeting*, (3rd Ordinary General Meeting of the Societas Europaea Herpetologica), 63–66.
- Bour, R. & Dubois, A. (1986). Nomenclature ordinale et familiale des Tortues (Reptilia). Note complémentaire. *Bulletin Mensuel de la Société Linnéenne de Lyon*, 55, 87–90.
- Bowen, B. W., Nelson, W. S. & Avise, J. C. (1993). A molecular phylogeny for marine turtles: trait mapping, rate assessment, and conservation relevance. *Proceedings of the National Academy of Sciences of the USA*, 90, 5574–5577.
- Bramble, D. M. (1974). Emydid shell kinesis: biomechanics and evolution. *Copeia*, 1974, 707–727.
- Busack, S. D. & Ernst, C. H. (1980). Variation in Mediterranean populations of *Mauremys* Gray 1869. *Annals of the Carnegie Museum of Natural History*, 49, 251–264.
- Caccone, A., Amato, G., Gratry, O. C., Behler, J. & Powell, J. R. (1999). A molecular phylogeny of four endangered Madagascar tortoises based on mtDNA sequences. *Molecular Phylogenetics and Evolution*, 12, 1–9.
- Ernst, C. H., Altenburg, R. G. M. & Barbour, R. W. (2000). *Turtles of the World*. World Biodiversity Database, CD-ROM Series, Windows, Version 1.2. Amsterdam: Biodiversity Center of ETI.
- Feldman, C. R. & Parham, J. F. (2002). Molecular phylogenetics of emydid turtles: taxonomic revision and the evolution of shell kinesis. *Molecular Phylogenetics and Evolution*, 22, 388–398.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.
- Fok, K. W., Wade, C. M. & Parkin, D. T. (2002). Inferring the phylogeny of disjunct populations of the azure-winged magpie *Cyanopica cyanus* from mitochondrial control region sequences. *Proceedings of the Royal Society of London, B* 269, 1671–1679.
- Fritz, U. (2001). *Mauremys* Gray, 1870 — Bachschildkröten. In U. Fritz (Ed.) *Handbuch der Amphibien und Reptilien Europas: Land- und Sumpfschildkröten* (pp. 35–42). Wiesbaden: AULA.
- Fritz, U. & Mendau, D. (2002). Ein Gattungsbastard zweier südostasiatischer Schildkröten: *Cuora amboinensis kamaroma* Rummeler & Fritz, 1991 × *Mauremys annamensis* (Siebenrock, 1903). *Salamandra*, 38, 129–134.
- Fritz, U. & Wischuf, T. (1997). Zur Systematik westasiatisch-südosteuropäischer Bachschildkröten (Gattung *Mauremys*) (Reptilia: Testudines: Bataguridae). *Zoologische Abhandlungen, Staatliches Museum für Tierkunde Dresden*, 49, 223–260.
- Gaffney, E. S. & Meylan, P. A. (1988). A phylogeny of turtles. In M. J. Benton (Ed.) *The Phylogeny and Classification of the Tetrapods*, Vol. 1 *Amphibians, Reptiles, Birds* (pp. 157–219). Oxford: Clarendon Press.
- Galgion, F. & Fritz, U. (2002). Captive bred hybrids between *Chinemys reevesii* (Gray, 1831) and *Cuora amboinensis kamaroma* Rummeler & Fritz, 1991. *Herpetozoa*, 15, 137–148.
- Gustincich, S., Manfioletti, G., del Sal, G., Schneider, C. & Carninci, C. (1991). A fast method for high-quality genomic DNA extraction from whole human blood. *Bio Techniques*, 11, 298–302.
- Hasegawa, M., Kishino, H. & Yano, K. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22, 160–174.
- Haskell, A. & Pokras, M. A. (1994). Nonlethal blood and muscle tissue collection from redbelly turtles for genetic studies. *Herpetological Review*, 25, 11–12.
- Hirayama, R. (1985). Cladistic analysis of batagurine turtles (Batagurinae: Emydidae: Testudinoidea): a preliminary result. In F. de Broin & E. Jiménez-Fuentes (Eds) *Studia Palaeoichnologia, Studia Geologica Salmanticensia*. Vol. I (pp. 141–157). Salamanca: Ediciones Universidad de Salamanca.
- Honda, M., Yasukawa, Y. & Ota, H. (2002a). Phylogeny of the Eurasian freshwater turtles of the genus *Mauremys* Gray, 1869 (Testudines), with special reference to a close affinity of *Mauremys japonica* with *Chinemys reevesii*. *Journal of Zoological Systematics and Evolutionary Research*, 40, 195–200.
- Honda, M., Yasukawa, Y., Hirayama, R. & Ota, H. (2002b). Phylogenetic relationships of the Asian Box turtles of the genus *Cuora* sensu lato (Reptilia: Bataguridae) inferred from mitochondrial DNA sequences. *Zoological Science*, 19, 1305–1312.
- Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Application Note*, 17, 754–755.
- Iverson, J. B. (1992). *A Revised Checklist with Distribution-Maps of the Turtles of the World*. Richmond, Indiana (privately printed).
- Iverson, J. B. & McCord, W. P. (1994). Variation in East Asian turtles of the genus *Mauremys*. *Journal of Herpetology*, 28, 178–187.
- Keller, C. & Busack, S. D. (2001). *Mauremys leprosa* (Schweigger, 1812) — Maurische Bachschildkröte. In U. Fritz (Ed.) *Handbuch der Amphibien und Reptilien Europas: Land- und Sumpfschildkröten* (pp. 57–88). Wiesbaden: AULA.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base pair substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: Molecular Evolutionary Genetics Analysis Software. *Bioinformatics*, 17, 1244–1245.
- de Lapparent de Broin, F. (2001). The European turtle fauna from the Triassic to the present. *Dumerilia*, 4, 155–217.
- Lattin, G. de (1967). *Grundriß der Zoogeographie*. Stuttgart: Fischer.
- Lecointre, G., Philippe, H., Van Le, H. L. & Le Guyader, H. (1994). How many nucleotides are required to resolve a phylogenetic

- problem? The use of a new statistical method applicable to available sequences. *Molecular Phylogenetics and Evolution*, 4, 292–309.
- Lenk, P., Fritz, U., Joger, U. & Wink, M. (1999). Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Molecular Ecology*, 8, 1911–1922.
- Lenk, P. & Wink, M. (1997). A RNA/RNA heteroduplex cleavage analysis to detect rare mutations in populations. *Molecular Ecology*, 6, 687–690.
- Loveridge, A. & Williams, E. E. (1957). Revision of the African tortoises and turtles of the suborder Cryptodira. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 79, 1–19.
- McCord, W. P. (1997). *Mauremys pritchardi*, a new batagurid turtle from Myanmar and Yunnan, China. *Chelonian Conservation Biology*, 2, 555–562.
- McCord, W. P., Iverson, J. B., Spinks, P. Q. & Shaffer, H. B. (2000). A new genus of geoemydid turtle from Asia. *Hamadryad*, 25, 20–24.
- McDowell, S. B. (1964). Partition of the genus *Clemmys* and related problems in the taxonomy of the aquatic Testudinidae. *Proceedings of the Zoological Society of London*, 143, 239–279.
- Merkle, D. A. (1975). A taxonomic analysis of the *Clemmys* complex (Reptilia: Testudines) utilizing starch gel electrophoresis. *Herpetologica*, 31, 162–166.
- Mindell, D. P., Sorenson, M. D., Dimcheff, D. E., Hasegawa, M., Ast, J. C. & Yuri, T. (1999). Interordinal relationships of birds and other reptiles based on whole mitochondrial genomes. *Systematic Biology*, 48, 138–152.
- Parham, J. F., Simison, W. B., Kozak, K. H., Feldman, C. R. & Shi, H. (2001). New Chinese turtles: endangered or invalid? A reassessment of two species using mitochondrial DNA, allozyme electrophoresis and known-locality specimens. *Animal Conservation*, 4, 357–367.
- Philippe, H., Germot, A. & Moreira, D. (2000). The new phylogeny of eukaryotes. *Current Opinion in Genetics and Development*, 10, 596–601.
- Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
- Pritchard, P. C. H. & McCord, W. P. (1991). A new emydid turtle from China. *Herpetologica*, 47, 139–147.
- Rodriguez, F. J., Oliver, L., Marín, A. & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, 142, 485–501.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.
- Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. (2002). TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics*, 18 (5), 502–504.
- Sedlag, U. (1995). *Urania Tierreich. Tiergeographie*. Berlin: Urania.
- Shaffer, H. B., Meylan, P. & McKnight, M. L. (1997). Tests of turtle phylogeny: molecular, morphological, and paleontological approaches. *Systematic Biology*, 46, 235–268.
- Shi, H. & Parham, J. F. (2001). Preliminary observations of a large turtle farm in Hainan Province, People's Republic of China. *Turtle and Tortoise Newsletter*, 3, 4–6.
- Sites, J. W., Bickham, J. W., Pytel, B. A., Greenbaum, I. F. & Bates, B. A. (1984). Biochemical characters and the reconstruction of turtle phylogenies: relationships among batagurine genera. *Systematic Zoology*, 33, 137–158.
- Strimmer, K. & von Haeseler, A. (1996). Quartet Puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Molecular Biology and Evolution*, 13, 964–969.
- Swofford, D. L. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sunderland, MA: Sinauer Associates.
- Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876–4882.
- Wermuth, H. & Mertens, R. (1961). *Schildkröten, Krokodile, Brückenechsen*. Jena: Fischer.
- Wermuth, H. & Mertens, R. (1977). Liste der rezenten Amphibien und Reptilien. Testudines, Crocodylia, Rhynchocephalia. *Das Tierreich*, 100, 1–174.
- Wink, M. (1995). Phylogeny of Old and New World vultures (Aves: Accipitridae and Cathartidae) inferred from nucleotide sequences of the mitochondrial cytochrome *b* gene. *Zeitschrift für Naturforschung*, 50c, 868–882.
- Wink, M., Guicking, D. & Fritz, U. (2001). Molecular evidence for hybrid origin of *Mauremys iversoni* Pritchard et McCord, 1991, and *Mauremys pritchardi* McCord, 1997 (Reptilia: Testudines: Bataguridae). *Zoologische Abhandlungen, Staatliches Museum für Tierkunde Dresden*, 51, 41–49.
- Wischuf, T. & Busack, S. D. (2001). *Mauremys rivulata* (Valenciennes in Bory de Saint-Vincent, et al. 1833) — Ostmediterrane Bachschildkröte. In U. Fritz (Ed.) *Handbuch der Amphibien und Reptilien Europas: Land- und Sumpfschildkröten* (pp. 89–110). Wiesbaden: AULA.
- Wischuf, T. & Fritz, U. (2001). *Mauremys caspica* (Gmelin, 1774) — Kaspische Bachschildkröte. In U. Fritz (Ed.) *Handbuch der Amphibien und Reptilien Europas: Land- und Sumpfschildkröten* (pp. 43–56). Wiesbaden: AULA.
- Wu, P., Zhou, K. & Yang, Q. (1999). Phylogeny of Asian freshwater and terrestrial turtles based on sequence analysis of 12S rRNA gene fragment. *Acta Zoologica Sinica*, 45, 260–267.
- Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution*, 39, 306–314.
- Yasukawa, Y., Hirayama, R. & Hikida, T. (2001). Phylogenetic relationships of Geoemydine turtles (Reptilia: Bataguridae). *Current Herpetology*, 20 (2), 105–133.
- Yasukawa, Y., Kamezaki, N. & Ichikawa, N. (1992). On hybrids between *Mauremys japonica* and *Chinemys reevesii*. *Japanese Journal of Herpetology*, 14 (4), 206–207.
- Yasukawa, Y., Ota, H. & Iverson, J. B. (1996). Geographic variation and sexual size dimorphism in *Mauremys mutica* (Cantor 1842) (Reptilia: Bataguridae), with description of a new subspecies from the Southern Ryukyus. *Zoological Science*, 13, 303–317.
- Zug, G. (1993). *Herpetology*. San Diego: Academic Press.