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Phylogenetic implications of karyotypic variation in the Batagurinae (Testudines: Emydidae)

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Abstract

The present study examined karyotypes of 16 genera and, along with previous reports, chromosomal data are now available for 18 of the 23 recognized batagurine genera. There are no karyotypic data available for the members of McDowell's (1964) *Hardella* complex. The *Batagur, Heosemys* and *Geoemyda* complexes retain the hypothesized primitive karyotype for the subfamily (2n = 52). All the genera in these three complexes have been examined except *Batagur* and *Annamemys*. The *Orlitia* complex is karyotypically distinct with 2n = 50 and the NOR located terminally on a large microchromosome. The genus *Malayemys* inclusion in the *Batagur* complex is not supported. *Malayemys* is characterized by a 2n = 50 karyotype, with the NOR located interstitially on a large microchromosome. The *Malayemys* complex is erected to contain this genus at a point intermediate between the *Orlitia* complex and the subfamily Emydinae. *Malayemys* and the emydines are karyotypically indistinguishable. The Neotropical genus *Rhinoclemmys* (*Geoemyda* complex) differs only slightly from the primitive batagurine karyotype in the position of the NOR. The species *R. funerea* and *R. punctularia* further differ in possessing one less metacentric macrochomosome. An interesting situation involves two subspecies of *R. punctularia*. The nominate subspecies is characterized by a 2n = 56 karyotype, while *R. p. melanosterna* reportedly has a 2n = 52 karyotype. Such a difference is interpreted as indicative of genetic differentiation between the two forms of a magnitude inconsistent with considering them as conspecific. Taken together with zoogeographic considerations, the karyotypic difference between the forms *R. p. punctularia* and *R. p. melanosterna* seem sufficient to warrant species distinction for *R. melanosterna* as previously suggested by Pritchard (1979b).

Introduction

Emydids constitute the largest family of turtles, containing nearly 40 percent of extant species. This large and diverse group of the most common and conspicuous turtles inhabiting the northern hemisphere remained largely unstudied on a global scale until recently. Not until 1964 were some relationships proposed which have been widely accepted. McDowell (1964) partitioned the group (which he considered a subfamily of the Testudinidae) into a primarily New World Emydinae and a primarily Old World Batagurinae. In addition, he allotted the genera in each subfamily into related groups, termed generic complexes. Bramble's (1974) study has been the only serious attempt to address generic relationships within the Batagurinae since McDowell (1964).

A rather liberal acceptance of genera characterizes the classification of batagurines recognized here (Table 1). Morphological studies have, in several cases, reached discordant conclusions regarding generic distinctions and phylogenetic relationships within the Batagurinae (Loveridge & Williams, 1957; Smith & James, 1958; McDowell, 1961, 1964; Parsons, 1968; Bramble, 1974; Albrecht, 1976). Recent listings of turtle species also differ in the generic designations of batagurines (Wermuth

Table 1. The genera of batagurines, after McDowell (1964), Bramble (1974), Wermuth and Mertens (1977), and Pritchard (1979a).

Batagur complex	*Geoemyda* complex
Batagur	*Annamemys*
Callagur	*Geoemyda*
Chinemys	*Mauremys*
Hieremys	*Melanochelys*
Kachuga	*Notochelys*
Malayemys	*Rhinoclemmys*
Ocadia	*Sacalia*
Hardella complex	*Heosemys* complex
Geoclemmys	*Cuora*
Hardella	*Cyclemys*
Morenia	*Heosemys*
	Pyxidea
Orlitia complex	
Orlitia	
Siebenrockiella	

& Mertens, 1977; Pritchard, 1979a).

Previous studies have noted a degree of variability in diploid number in emydids not found among other cryptodiran families (Stock, 1972; Bickham, 1975; Bickham & Baker, 1976a, 1979; Bickham & Carr, 1983; Killebrew, 1977). The Emydinae is karyotypically homogeneous (Bickham & Carr, 1983), but the Batagurinae is variable. A diploid number of 52 appears characteristic of most batagurines (Table 2), but there are several reports of 2n = 50 in some species and two reports of 2n = 56 in *Rhinoclemmys punctularia.* An exploration and explication of this variability in a phylogenetic context is the subject of this account.

Material and methods

Standard karyotypes were routinely prepared

Table 2. Summary of the karyotypic data considered in the present study.

Taxon	2n	A:B:C	Numbers of Specimens Examined	Figure			
			male:female:juvenile	Std.	G-band	C-band	NOR
Callagur borneoensis	52	9:5:12	0:0:1	NS	2c	NS	NS
Chinemys kwangtungensis	52	9:5:12	0:0:1	2a	–	1c	–
C. reevesii	52	9:5:12	0:0:1	–	+	–	–
Cuora ambionensis	52	9:5:12	0:1	8a	8b	NS	NS
C. trifasciata	52	9:5:12	1:0	8c	–	–	–
Cyclemys dentata	52	9:5:12	0:0:1	–	–	+	–
Heosemys grandis	52	9:5:12	0:1	7c	–	–	–
H. spinosa	52	9:5:12	0:1	NS	–	–	NS
Hieremys annandalii	52	9:5:12	0:0:1	NS	2b	NS	NS
Malayemys subtrijuga	50	8:5:12	0:0:1	NS	9b	–	10c
Mauremys japonica	52	9:5:12	1:0	3a	–	–	–
Melanochelys trijuga	52	9:5:12	1:1	3b	–	–	–
Notochelys platynota	52	9:5:12	0:0:1	3c	–	–	–
Ocadia sinensis	52	9:5:12	0:2	1a	1b	NS	1d
Orlitia borneensis	50	8:5:12	0:1	NS	9a	NS	10b
Pyxidea mouhotii	52	9:5:12	0:1	7b	–	–	NS
Rhinoclemmys areolata	52	9:5:12	0:1	4c	NS	6c	6a
R. funerea	52	8:5:13	1:0	5a	NS	NS	NS
R. pulcherrima incisa	52	9:5:12	2:0	4b	7a	–	NS
R. p. manni	52	9:5:12	3:4	4a	NS	NS	6b
R. punctularia punctularia	56	8:5:15	2:1	5b	x	x	–
R. rubida	52	9:5:12	0:1:1	5c	–	–	–
Sacalia bealei	52	9:5:12	1:0	y	x	x	–
Siebenrockiella crassicollis	50	8:5:12	6:4	*	*	*	10a

* See figures in Carr and Bickham (1981).
+See figures in Haiduk and Bickham (1982).
x See figures in Bickham and Baker (1976a).
y See figures in Bickham (1975).
NS Not shown.

directly from spleen according to the procedure of Bickham (1975), with modifications as noted in Carr and Bickham (1981). Cells utilized for differential staining techniques were obtained from heart fibroblast cell cultures as described in Sites *et al.* (1979). The modifications of Seabright's (1971) and Sumner's (1972) techniques as described by Sites *et al.* (1979) were used for G-banding and C-banding, respectively. The Ag-AS technique of Goodpasture and Bloom (1975) was employed for staining nucleolus organizer regions (NOR).

The following specimens examined are deposited in the Texas Cooperative Wildlife Collection of Texas A&M University: *Callagur borneoensis,* TCWC 58357; *Chinemys kwangtungensis,* TCWC 60716; *Chinemys reevesii,* TCWC 56736; *Cuora amboinensis,* TCWC 56951; *Cuora trifasciata,* TCWC 58349; *Cyclemys dentata,* TCWC 56965; *Heosemys grandis,* TCWC 58350; *Heosemys spinosa,* TCWC 56953; *Hieremys annandalii,* TCWC 56935, 56959; *Malayemys subtrijuga,* TCWC 58364; *Mauremys japonica,* TCWC 60719; *Notochelys platynota,* TCWC 58366; *Ocadia sinensis,* TCWC 56955, 57879; *Pyxidea mouhotii,* TCWC 58358; *Rhinoclemmys areolata,* TCWC 57878; *R. funerea,* TCWC 58337; *R. rubida,* TCWC 58355; *R. punctularia punctularia,* TCWC 58616–17, 58627; *R. pulcherrima incisa,* TCWC 55016, 58375; *R. p. manni,* TCWC 56862–63, 56911–14, 56999; *Sacalia bealei,* TCWC 60718; *Siebenrockiella crassicollis,* TCWC 56942–43, 58204, 58344–47, 58360, 58648. Specimens examined which are deposited in other collections include: *Melanochelys trijuga,* University of Utah 17502; *Orlitia borneensis,* E. O. Moll, private collection; *Rhinoclemmys rubida,* Los Angeles County Museum 131362; *Siebenrockiella crassicollis,* LACM 116540.

The terminology for centromere position used herein is that of Bickham (1975) as adapted from Levan *et al.* (1964). The karyotype is divided into three groups based on relative size and centromere position. Group A chromosomes are characterized as either metacentric or submetacentric macrochromosomes. Group B macrochromosomes are either subtelocentric or telocentric. The microchromosomes of Group C are all so small that it is difficult to consistently determine centromere position, or even homologs in G-band preparations, with any certainty.

Results

A summary of the karyotypic data for each species is presented in Table 2, as are references to the illustrations (Figs. 1–10). In general, groups of genera were found to be karyotypically homogeneous and therefore the accounts which follow are organized by generic complex. The arrangement of chromosome pairs in each of the aforementioned three groups is presented in the form A:B:C.

Batagur complex

The genera *Callagur, Chinemys, Hieremys, Malayemys,* and *Ocadia* were included in the present study. Several species of *Kachuga* have previously been studied by other workers and some reference to that work will be made. *Batagur* is the only genus in this complex which has not been studied.

The species *Callagur borneoensis, Chinemys kwangtungensis, C. reevesii, Hieremys annandalii,* and *Ocadia sinensis* all possess indistinguishable 2n=52 (9:5:12) standard karyotypes (Figs. 1a, 2a). G-banded karyotypes were obtained from *Hieremys* (Fig. 2b), *Ocadia* (Fig. 1b), and *C. reevesii* (Bickham *et al.,* 1980) and are indistinguishable as well. It is the same G-band pattern as that illustrated by Bickham and Baker (1976a) for *Sacalia bealei.* C-bands of *Ocadia* and *C. kwangtungensis* (Fig. 1c) show the ninth group A macrochromosome pair to be largely heterochromatic, as is also the case in *Sacalia* (Bickham & Baker, 1976a). Silver staining shows the single pair of NORs to be located telomerically on the smallest group A macrochromosome pair in *Callagur* and *Ocadia* (Fig. 1d).

Unlike the other members of the *Batagur* complex, *Malayemys* has a diploid number of 50 (8:5:12), with one less pair of group A macrochromosomes. The G-banding pattern of this species (Fig. 9b) is the same as the emydine genera *Graptemys, Pseudemys,* and *Terrapene* (Bickham & Baker, 1979), which is identical to the macrochromosomes of the batagurines mentioned above (excepting pair 9A). The NOR of *Malayemys* appears on one of the largest microchromosome pairs proximal to the centromere (Fig. 10c). Based upon gross morphology, G-band, and NOR location, no karyotypic distinction between *Malayemys* and emydines (Bickham & Baker, 1976a, 1979 and unpublished) can be made.

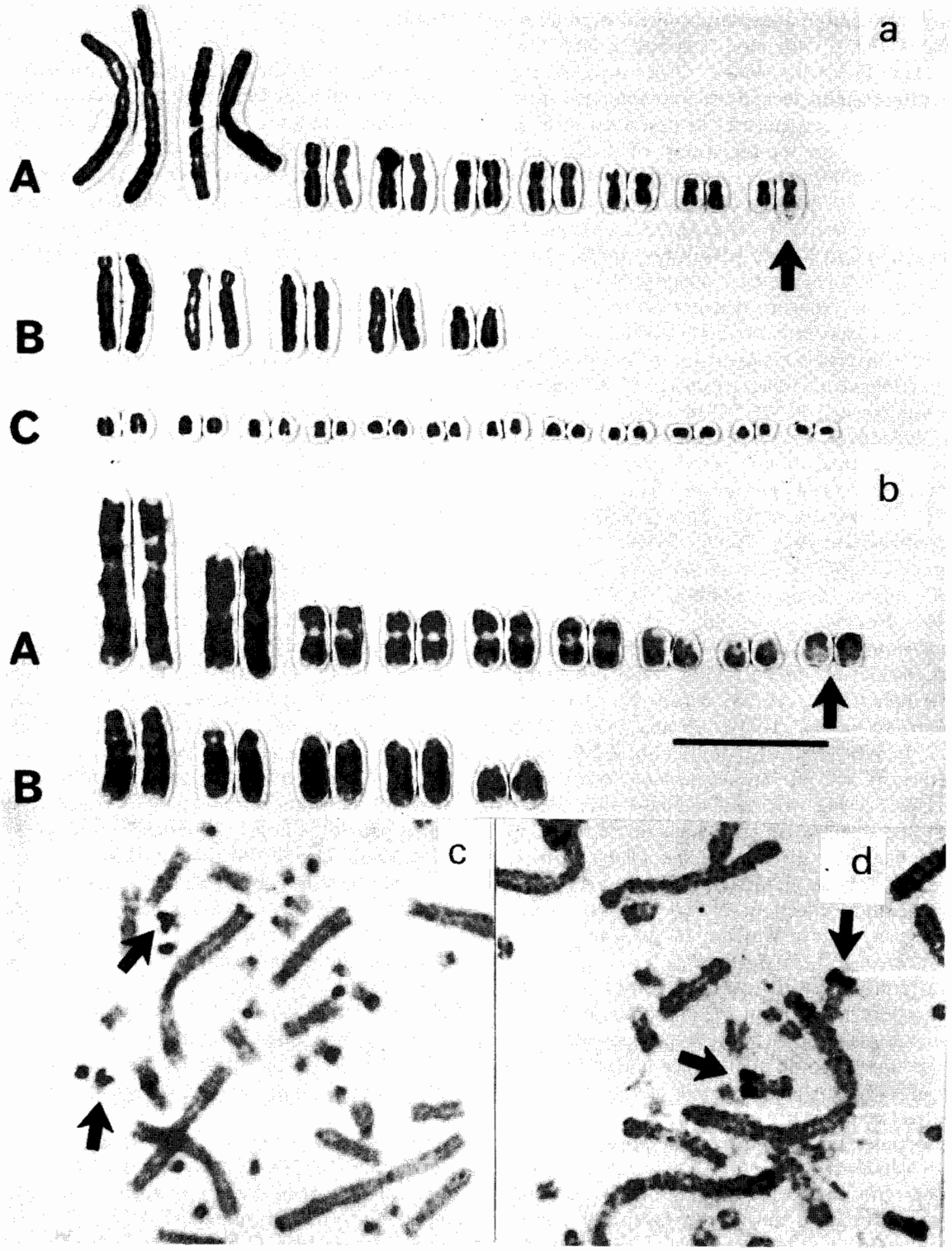

Fig. 1. (a) Standard karyotype of *Ocadia sinensis.* Arrows in this and all subsequent figures identify the NOR-bearing chromosome, if identifiable. Chromosomes are arranged into groups A:B:C as described in the text. (b) G-band karyotype of *O. sinensis.* Bar, in this and subsequent figures, is 10 microns. (c) C-banded partial metaphase of *Chinemys kwangtungensis.* (d) Silver stained partial metaphase of *O. sinensis.*

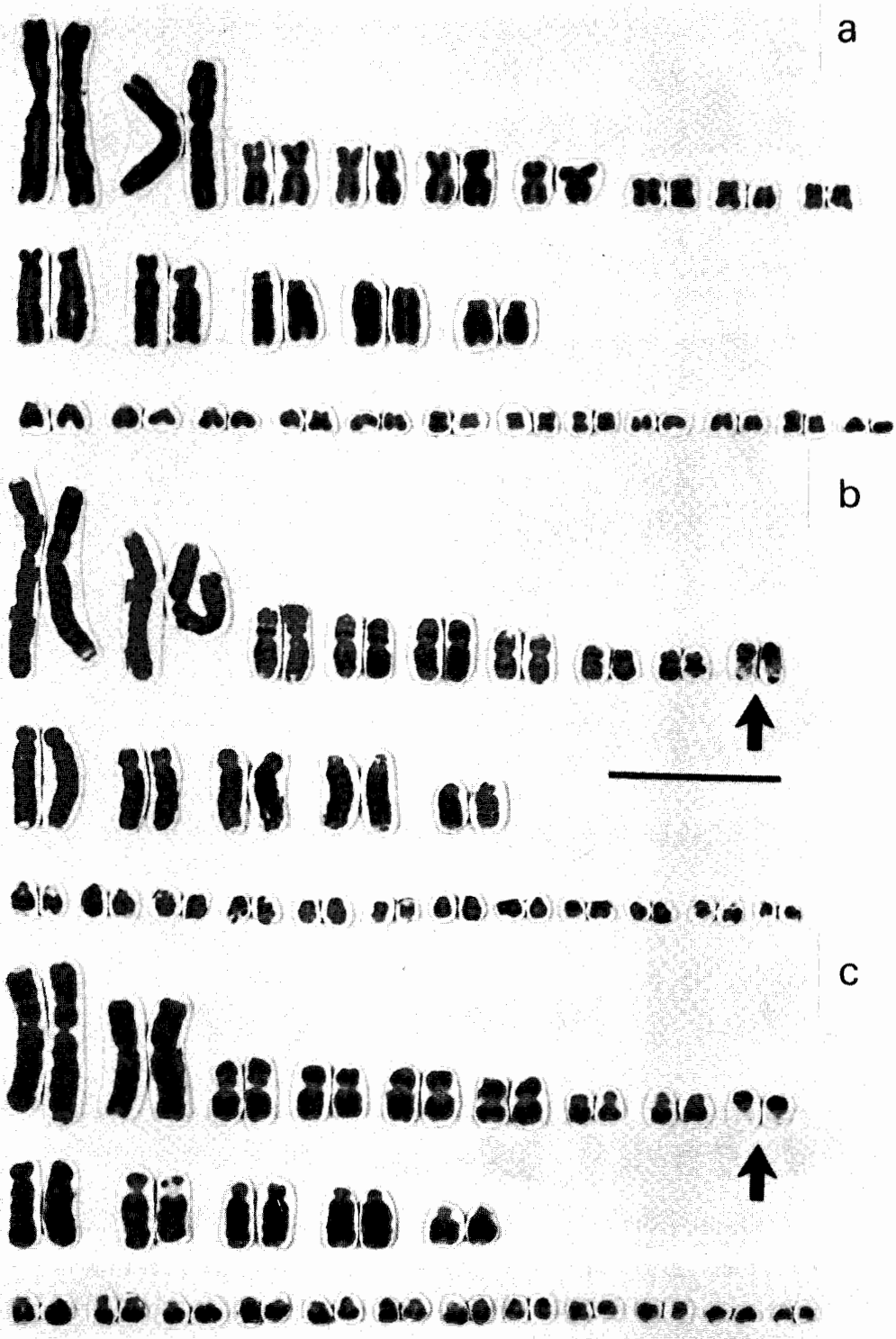

Fig. 2. (a) Standard karyotype of *Chinemys kwangtungensis.* (b) G-band karyotype of *Hieremys annandalii.* (c) G-band karyotype of *Callagur borneoensis.*

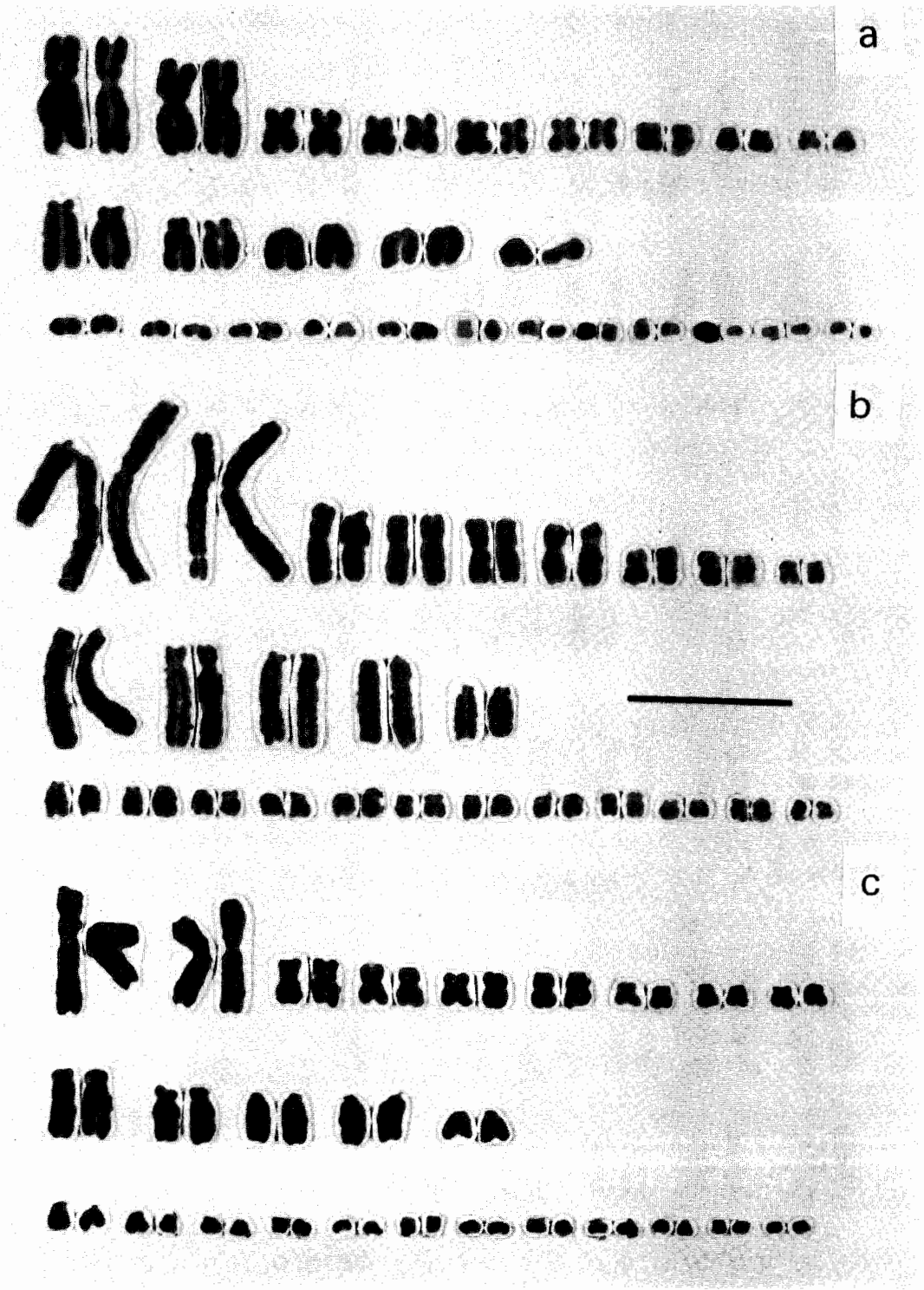

Fig. 3. Standard karyotypes of (a) *Mauremys japonica,* (b) *Melanochelys trijuga,* (c) *Notochelys platynota.*

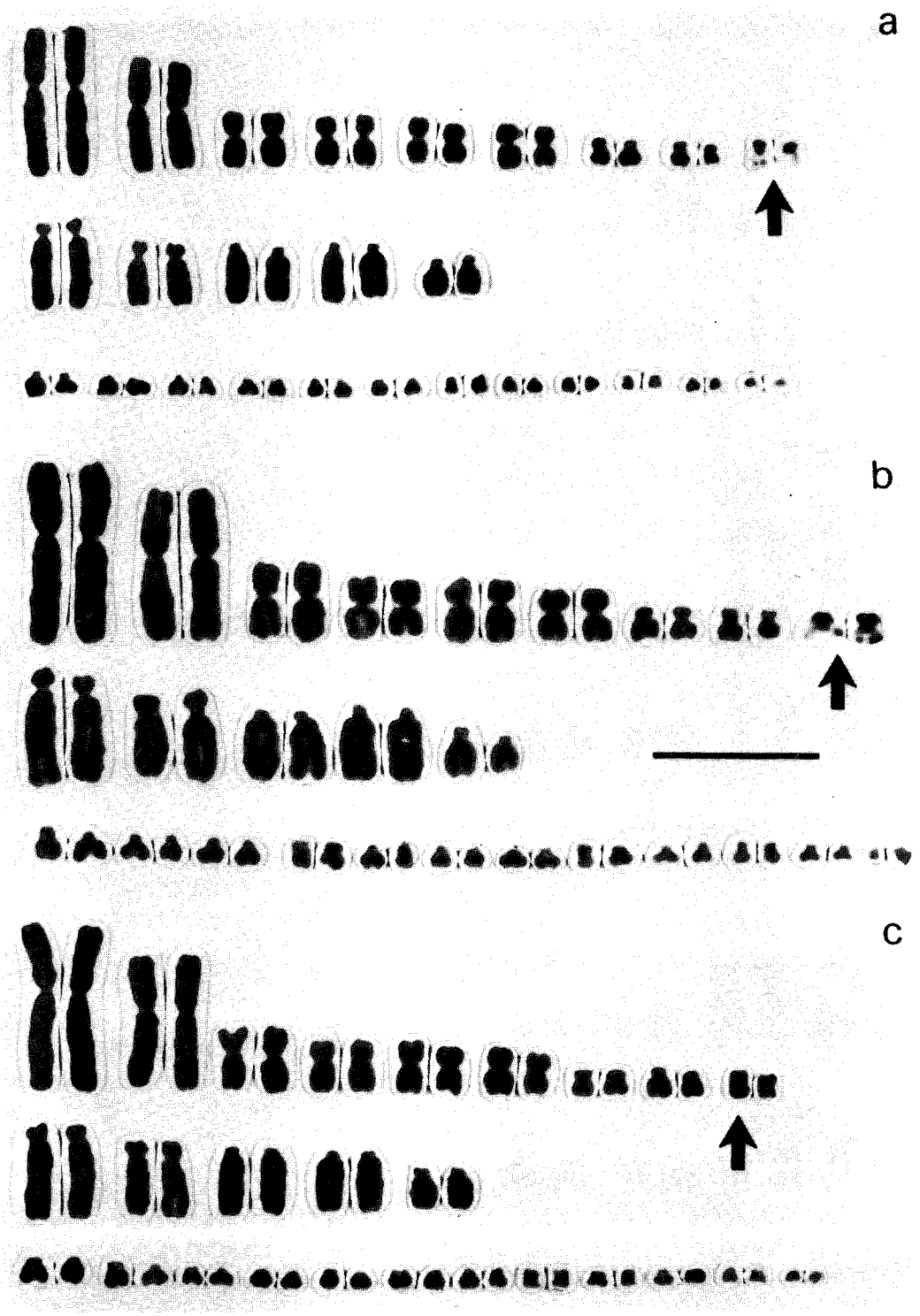

Fig. 4. Standard karyotypes of (a) *Rhinoclemmys pulcherrima manni,* (b) *R. p. incisa,* (c) *R. areolata.*

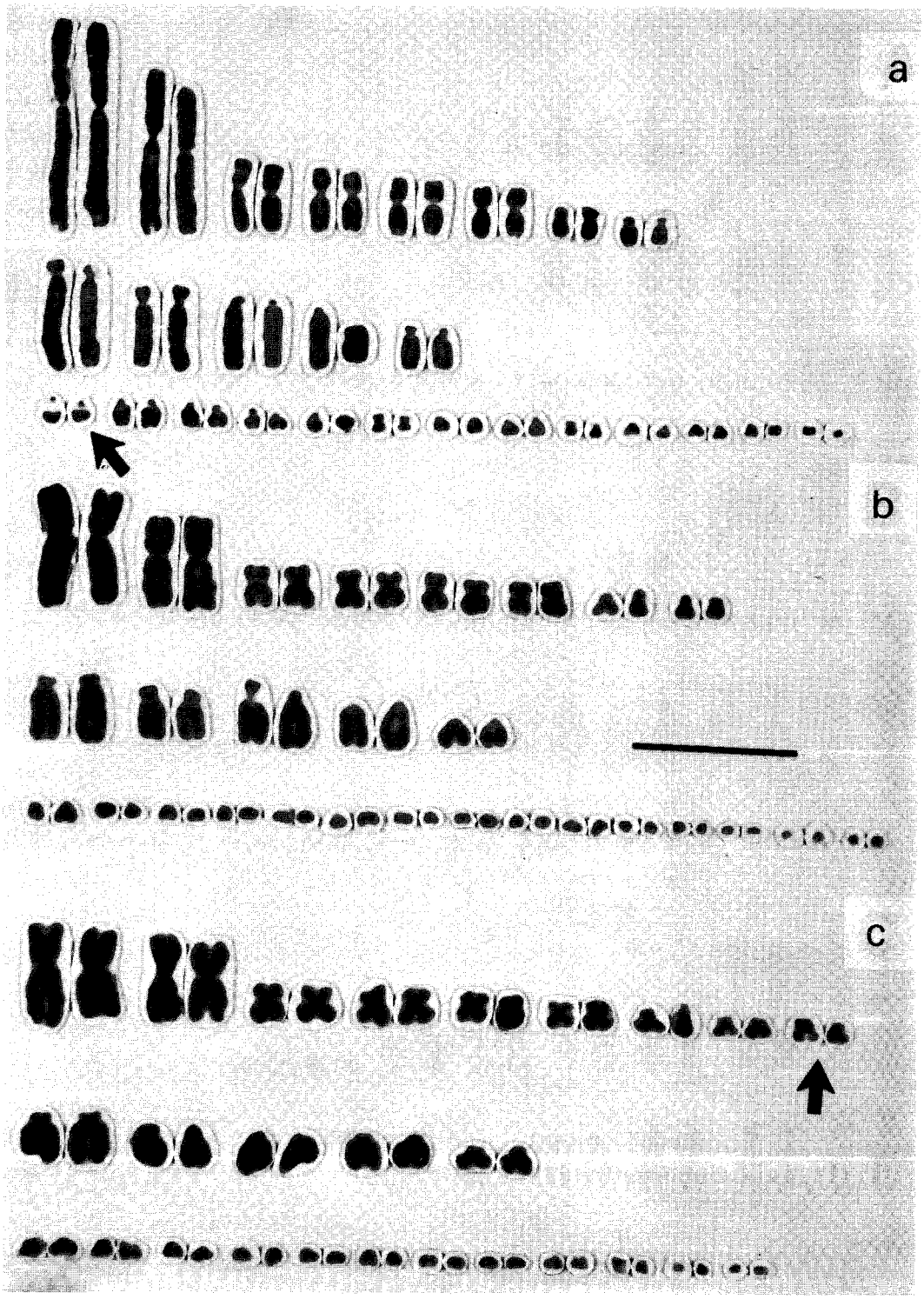

Fig. 5. Standard karyotypes of (a) *Rhinoclemmys funerea,* (b) *R. punctularia punctularia,* (c) *R. rubida.*

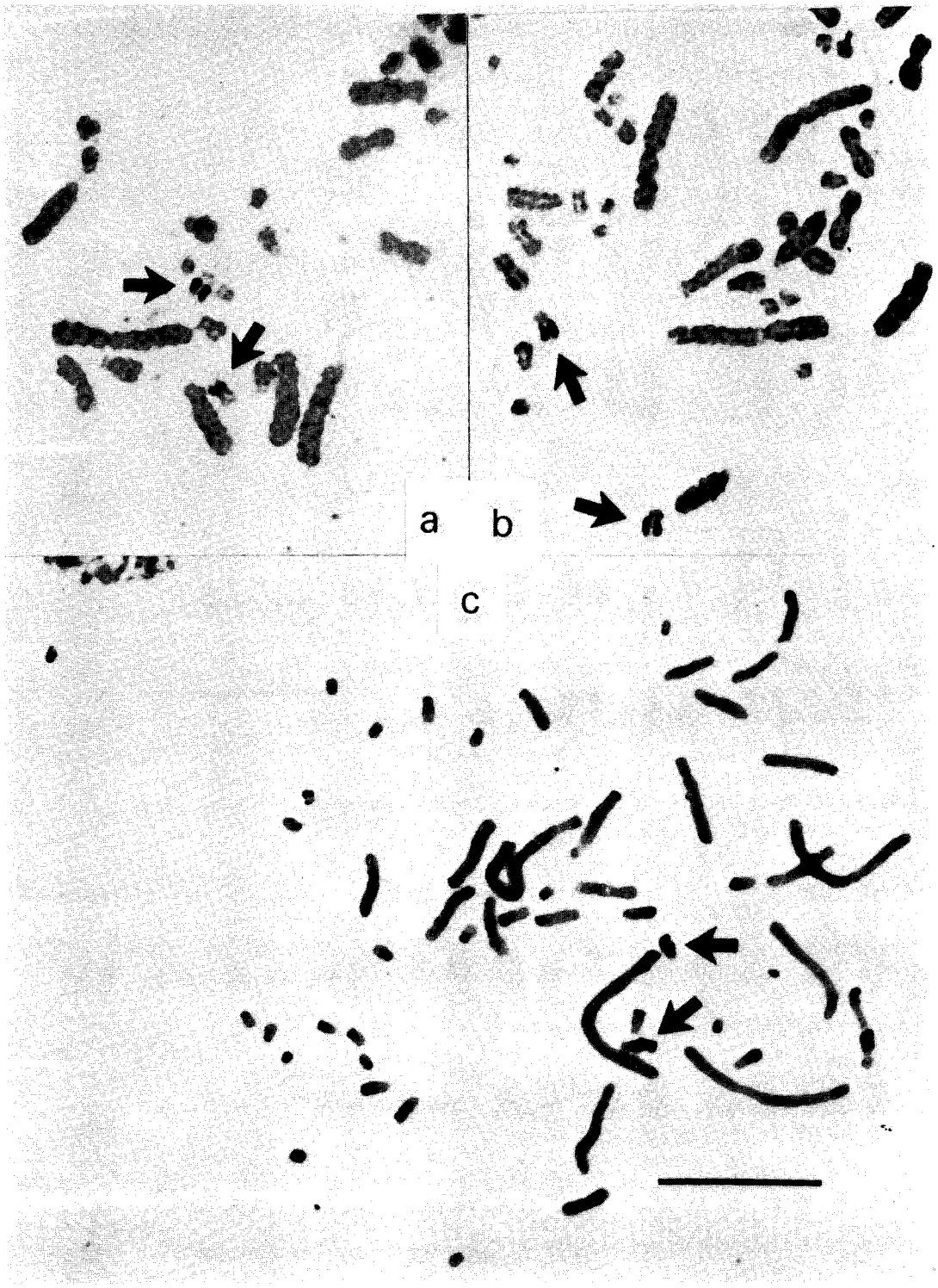

Fig. 6. Silver stained partial metaphase plates of (a) *Rhinoclemmys areolata* and (b) *R. pulcherrima manni.* (c) C-banded partial metaphase plate of *R. areolata.*

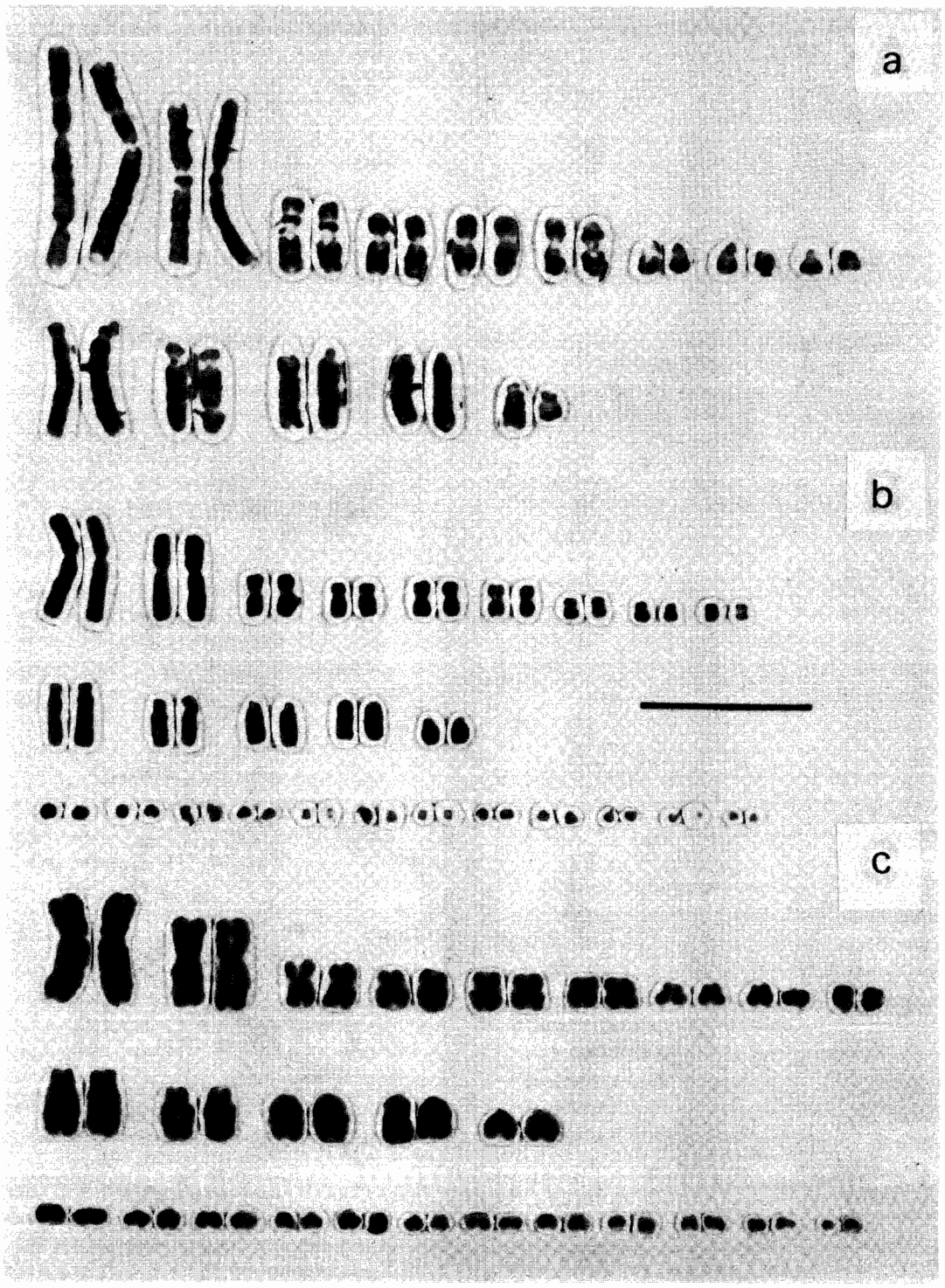

Fig. 7. (a) G-banded macrochromosomes of *Rhinoclemmys pulcherrima incisa.* Standard karyotypes of (b) *Pyxidea mohoutii* and (c) *Heosemys grandis.*

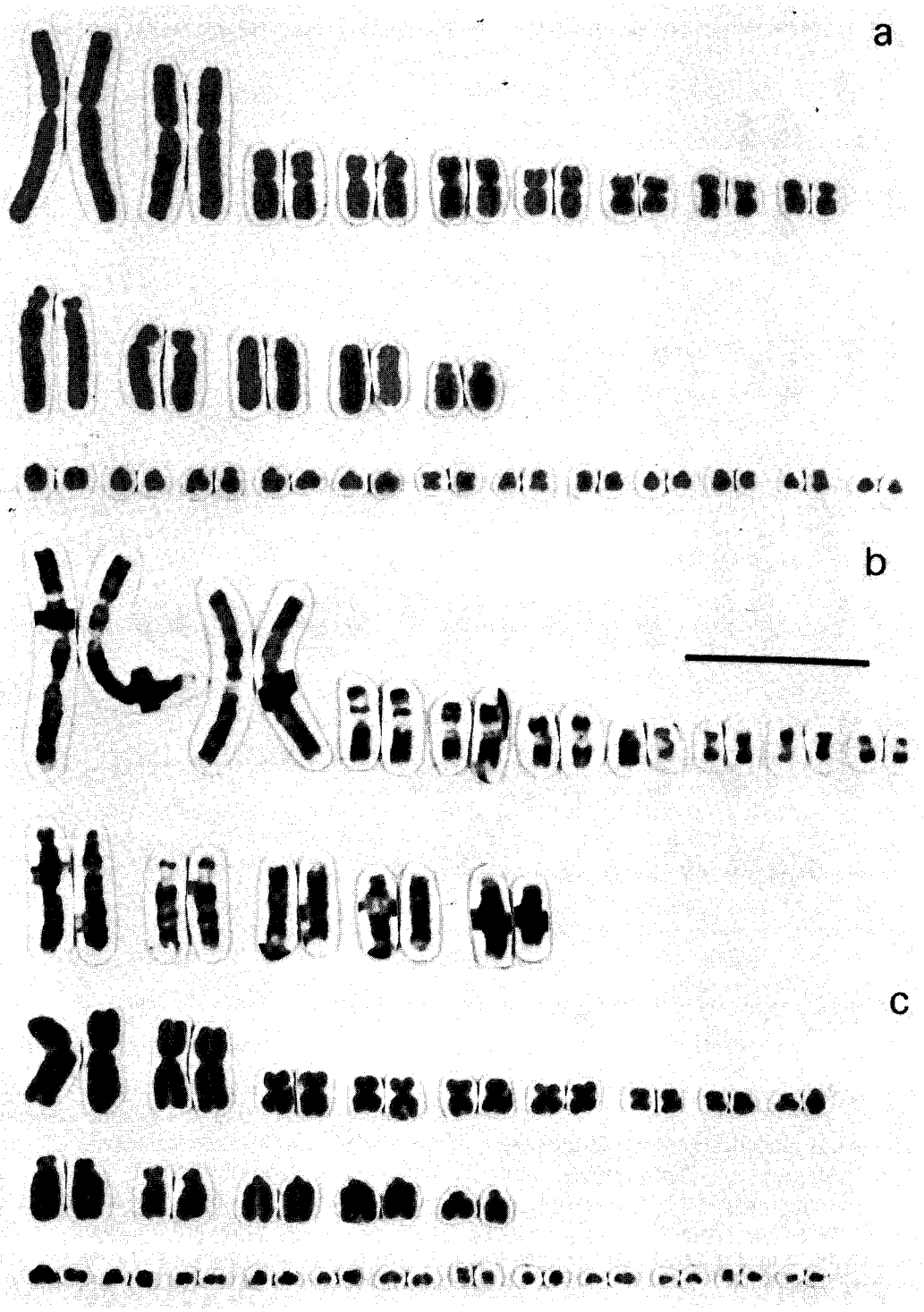

Fig. 8. Standard karyotype (a) and G-band karyotype (b) of *Cuora amboinensis.* (c) Standard karyotype of *C. trifasciata.*

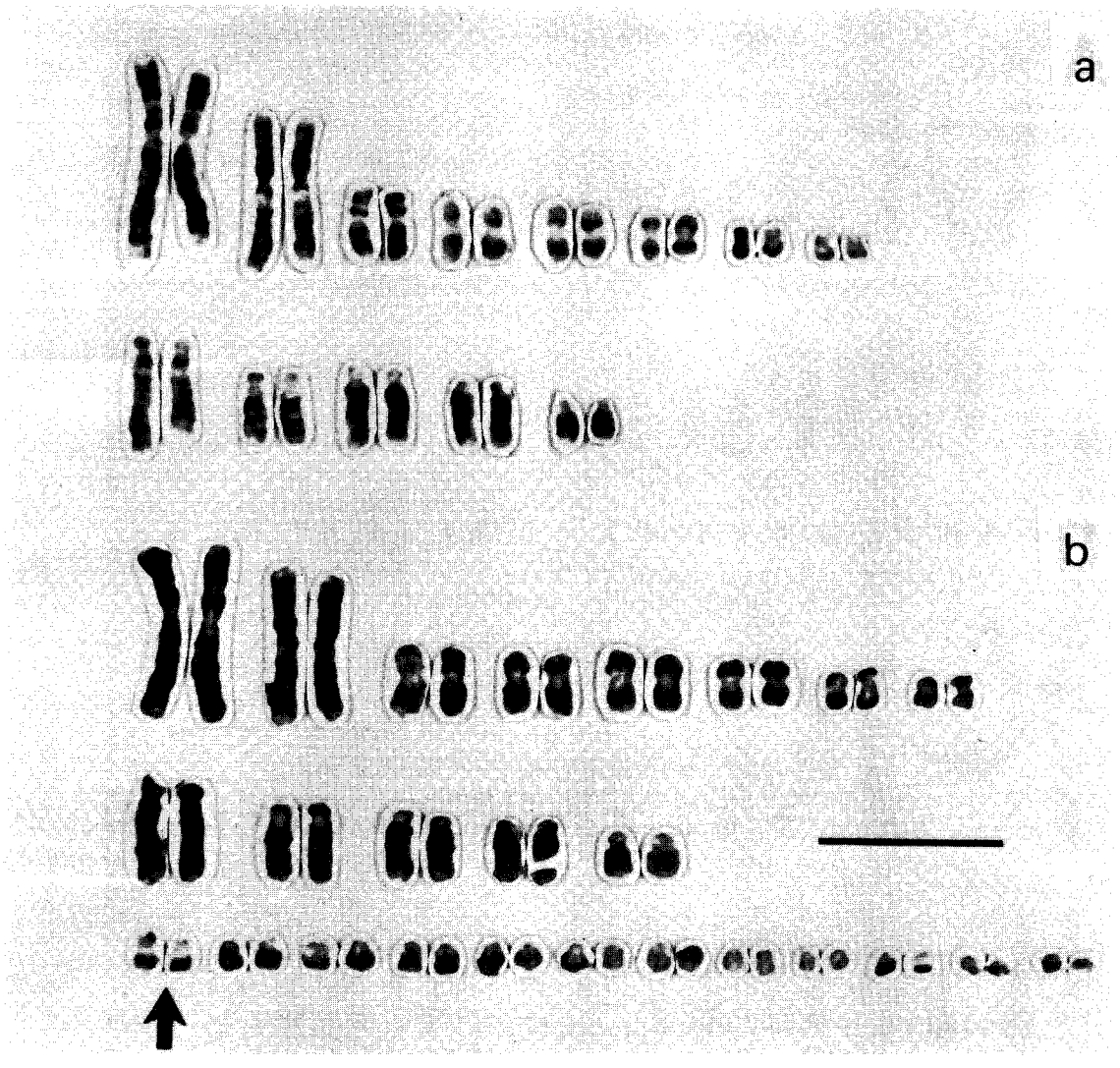

Fig. 9. (a) G-banded macrochromosomes of *Orlitia borneensis.* (b) G-band karyotype of *Malayemys subtrijuga.*

Geoemyda complex

The present study includes karyotypic data on *Melanochelys, Mauremys, Notochelys,* and *Rhinoclemmys.* Comparative data are available in the literature for the genera *Geoemyda* and *Sacalia* (see references in Bickham & Carr, 1983). *Annamemys* has not been examined.

Mauremys (Fig. 3a), *Melanochelys trijuja* (Fig. 3b), *Notochelys* (Fig. 3c), and most *Rhinoclemmys* (Figs. 4 and 5) have the same gross morphology that characterizes most of the *Batagur* complex, namely 2n=52 (9:5:12). Reference to the literature reveals that the genera *Geoemyda* and *Sacalia* possess this same karyotype. The data available at present do not indicate a distinction in the number of macrochromosomes between some members of the genus *Rhinoclemmys* and the Old World batagurines with 2n=52 as was interpreted by Bickham and Baker (1976a). The taxa *R. p. incisa, R. p. manni, R. areolata,* and *R. rubida* possess karyotypes with 2n=52, (9:5:12) (Figs. 4 and 5). However, the smallest group A pair (9A) possesses a distinct, interstitial, secondary constriction that

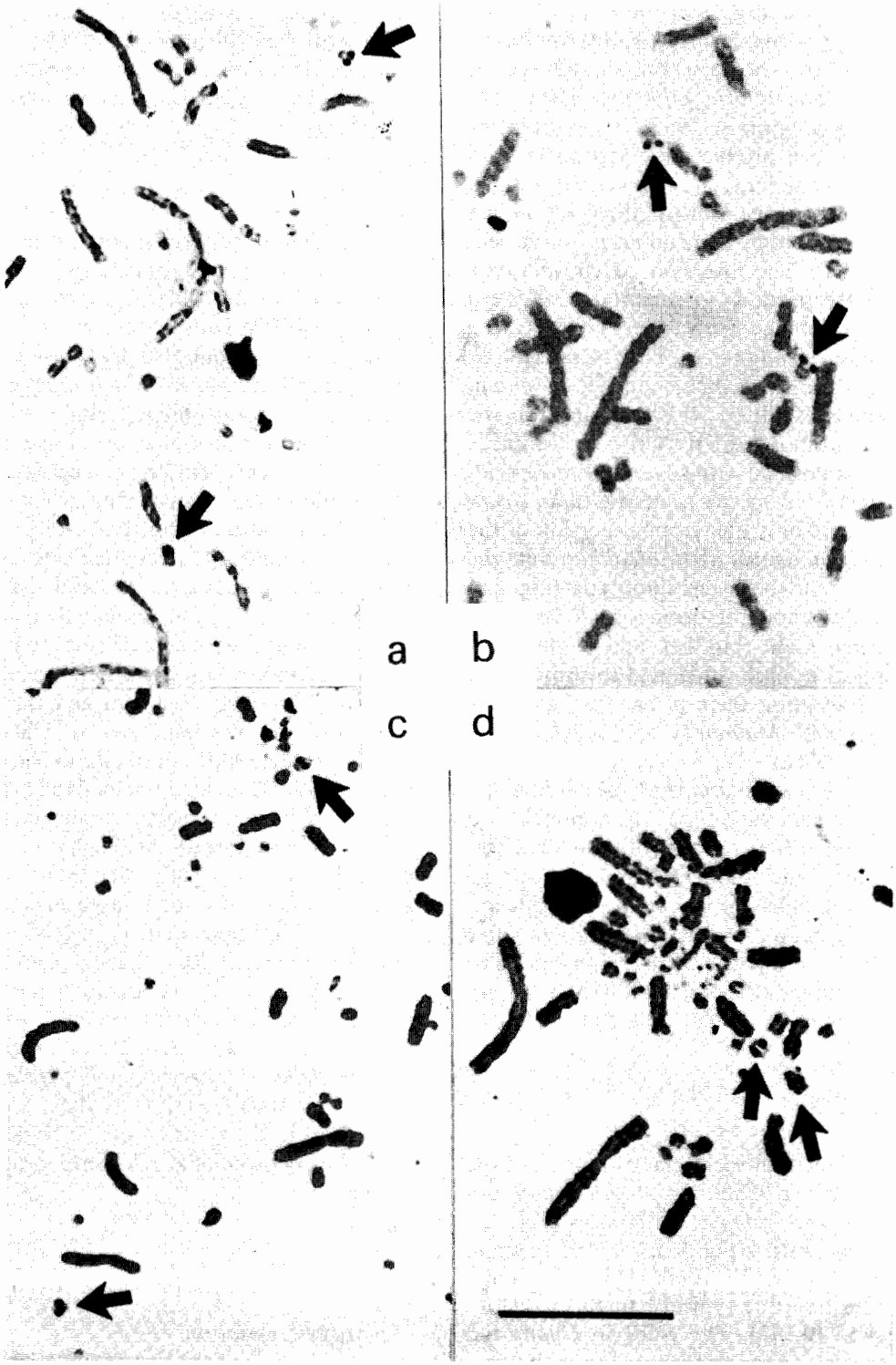

Fig. 10. Silver stained partial metaphases of (a) *Siebenrockiella crassicollis,* (b) *Orlitia borneensis,* (c) *Malayemys subtrijuga,* and (d) *Clemmys guttata,* Emydinae.

differentiates the karyotypes of these species from the presumed primitive 2n=52 (9:5:12) karyotype (e.g., Fig. 1a). There is no detectable secondary constriction in *R. punctularia punctularia* (Fig. 5b) and only 8 pairs in group A. We have not yet located the NOR (which produces the secondary constriction) by silver staining in *R. punctularia.* In *R. funerea* the secondary constriction is on an acrocentric microchromosome adjacent to the centromere (Fig. 5a). The inversion of chromosome 9A of *R. pulcherrima, R. rubida,* and *R. areolata* could account for the acrocentric NOR-bearing chromosome of *R. funerea.* The NORs of *R. areolata* (Fig. 6a), *R. p. incisa,* and *R. p. manni* (Fig. 6b) were located, by silver staining, on the long arm of chromosome 9A. Virtually the entire long arm stains positive with silver in these species and this differs from species such as *Ocadia sinensis* (Fig. 1d) where only the terminal portion of the long arm stains. C-bands of *R. areolata* reveal the long arm of 9A to be heterochromatic (Fig. 6c). The G-banded macrochromosomes of *R. areolata, R. pulcherrima incisa* (Fig. 7a), and *R. p. manni* appear identical to each other and to those of *R. punctularia* (excepting there is one less group A pair in this species), *Mauremys,* and *Sacalia* (Bickham & Baker, 1976a).

Rhinoclemmys punctularia is of special note because of its divergent karyotype. The nominal form *R. p. punctularia* differs from all other *Rhinoclemmys* examined, including *R. p. melanosterna* (Killebrew, 1977), in having two extra pairs of heterochromatic microchromosomes. Studies now show that *R. p. punctularia* is 2n=56 in disjunct parts of its range, i.e. Trinidad (Bickham & Baker, 1976a, b), Venezuela (present account), and Brazil (Barros *et al.,* 1975).

Heosemys complex

Data are included for representatives of all of the genera in this group (Table 2). *Pyxidea mouhotii* (Fig. 7b), *Heosemys spinosa, H. grandis* (Fig. 7c), *Cuora amboinensis* (Fig. 8a), *C. trifasciata* (Fig. 8c), and *Cyclemys dentata* (Haiduk & Bickham, 1982) all have the typical batagurine karyotype of 2n=52 (9:5:12). The NOR in *Cuora* and *Pyxidea* is located on the smallest group A chromosome pair, the same chromosome pair that is largely heterochromatic in *Cyclemys* (Haiduk & Bickham, 1982). G-bands of *Cuora amboinensis* (Fig. 8b) show identical banding patterns of the macrochromosomes as the presumed primitive 2n=52 karyotype, such as in *Ocadia sinensis* (Fig. 1b).

Orlitia complex

The diploid number is 50 and the arrangement 8:5:12 in both *Orlitia* and *Siebenrockiella* (Fig. 9a; Carr & Bickham, 1981). A sex chromosome heteromorphism involving the second group B chromosome pair was found in *Siebenrockiella* (Carr & Bickham, 1981). Males of *Siebenrockiella* are heteromorphic for centromere position on this chromosome and the females are homomorphic (an XX/XY system). Differences between the X and the Y and a discussion of their evolution is presented in Carr and Bickham (1981). The subtelocentric X chromosome of *Siebenrockiella* appears completely homologous in G-band pattern to the homomorphic second group B pair of *Orlitia* (Fig. 9a) and other emydids (Fig. 19 in Bickham & Baker, 1976a). A possible difference in G-band pattern mentioned by Bickham and Baker (1976a) is probably due to comparison of chromosomes of different degrees of contraction. The NOR in *Siebenrockiella* is located telomerically on one of the larger pairs of microchromosomes (Fig. 10a), which is also largely heterochromatic (Fig. 2 in Carr & Bickham, 1981). The NOR of *Orlitia* appears telomerically on a large microchromosome pair, as in *Siebenrockiella* (Fig. 10b). *Orlitia* differs from *Siebenrockiella* in having the centromere of the second group A macrochromosome pair within a dark G-band region (as it is in all other emydids), rather than within a light G-band region as in *Siebenrockiella.* The karyotype of *Orlitia* differs from that of *Malayemys* (Fig. 10c) and emydines (Fig. 10d) in the position of the NOR on the large microchromosome (i.e. telomeric rather than interstitial).

Discussion

Karyotypic variation

Some karyotypic data are available in the literature for 13 of the 23 batagurine genera, mostly con-

cerning gross morphology (Bickham & Carr, 1983). The present study has examined 24 species and subspecies in 16 genera, bringing the total number of genera for which karyotypic data are available to 18. Discrepancies between literature reports of diploid numbers and results obtained during this study are not usually considered to represent intraspecific karyotypic variation. It seems most likely that the discrepancies result from different levels of resolution achieved by the investigators, as all of the differences involve the number of microchromosomes.

The genera for which there are currently no karyotypic data available are *Annamemys, Batagur,* and those of the *Hardella* complex. Bickham (1975) and Bickham and Baker (1976a) considered the 2n=52 (9:5:12) karyotype exemplified by *Mauremys* and *Sacalia* to be primitive for the Batagurinae and the entire family. Essentially the same conclusion is implied or explicit in Killebrew (1977) and Dowler and Bickham (1982). In addition, the same karyotype is shared with some testudinids, a relationship interpreted as evidence for the hypothesized origin of tortoises from a 'proto-emydid' stock (Loveridge & Williams, 1957; Bickham & Baker, 1976a; Killebrew & McKown, 1978; Dowler & Bickham, 1982). Eleven other genera of batagurines have this same karyotype (Table 2; Bickham & Carr, 1983). The genera *Geoemyda* and *Kachuga* should also be considered among this group possessing the primitive karyotype (Nakamura, 1949; Singh, 1972; Stock, 1972; Killebrew, 1977; DeSmet, 1978). All genera for which banding data are currently available show essentially identical G-band patterns with the NOR located on the largely heterochromatic ninth group A macrochromosome pair.

Three genera of Asian batagurines have 2n=50 karyotypes with eight group A and five group B pairs. Gross morphology of the chromosomes in *Malayemys, Orlitia,* and *Siebenrockiella* appears identical to that of the 2n=50 emydines, excepting the sex chromosomes of *Siebenrockiella.* The presence or absence of a sex chromosome system in *Orlitia* is unproved because we examined only a single juvenile (female?) specimen. The G-band patterns of *Malayemys* and *Orlitia* are the same as the emydines. Besides differences associated with the sex chromosome pair in *Siebenrockiella,* the second group A pair in *Siebenrockiella* has the centromere located in a G-band negative region whereas in other emydids it is located in a G-positive region. The NOR of *Orlitia* and *Siebenrockiella* is located telomerically on a large heterochromatic microchromosome. In *Malayemys* (and emydines) the NOR is located interstitially. We have been unable to detect any karyotypic differences between the batagurine *Malayemys* and the emydines.

Five species of the Neotropical genus *Rhinoclemmys* have now been examined karyotypically. All but *R. p. punctularia* and *R. funerea* have been shown to be nearly identical to the presumed primitive 2n=52 (9:5:12) karyotype. The macrochromosomes of *Rhinoclemmys* species appear indistinguishable from those of other emydids except that the NOR appears to be in a slightly different position compared to the Asian 2n=52 genera. *R. funerea* and *R. p. punctularia* have diverged from the modal *Rhinoclemmys* karyotype in having one less group A macrochromosome pair (8 rather than 9). Also, *R. p. punctularia* has two extra pairs of heterochromatic microchromosomes.

Rhinoclemmys is the only genus of cryptodiran turtles in which varaition in diploid number has been demonstrated (Bickham & Baker, 1979). It is of interest that Killebrew (1977) reported 2n=52 in *R. p. melanosterna.* Since Boulenger (1889), the form *melanosterna* has been considered a subspecies *R. punctularia.* Ernst's (1978) recent revision of the genus also considered it as such. Pritchard (1979b) regarded *melanosterna* to be a full species. *R. p. melanosterna* is found in eastern Panama, northern and western Colombia, and northwestern Ecuador. It is apparently geographically isolated from the neighboring *R. p. diademata* of northeastern Colombia and northwestern Venezuela by the Sierra de Perija. The nominal subspecies, *R. p. punctularia,* occurs in northeastern Venezuela, on Trinidad, and throughout the Guianan region into Amazonian Brazil. The forms *punctularia* and *diademata* are separated by the Sierra de Merida and central llanos of Venezuela (Pritchard, 1979b; Ernst, 1981). The great disjunction of range between the forms *punctularia* and *melanosterna,* taken together with the cytogenetic distinction between the two, leads us to agree with Pritchard (1979b) in considering *melanosterna* a distinct species. This view hinges, of course, on the accuracy of Killebrew's (1977) report of 2n=52 for *R. p. melanosterna.* Examination of the form *diademata* would be of interest because of its geo-

graphic position relative to *melanosterna* and *punctularia.*

Phylogenetic implications

Most batagurine genera have retained the primitive 2n=52 (9:5:12) karyotype with the NOR located on the smallest group A macrochromosome, a karyotype shared with some testudinids (Dowler & Bickham, 1982). In other emydids the NOR is located on a large microchromosome. A dichotomy occurs in which the 2n=50 karyotype is derived by loss of the 9th group A chromosome pair which formerly contained the NOR. It cannot be ruled out for certain that the NOR is not located on the same chromosome in both (that is, the 2n=52 and 2n=50 karyotypes) with that chromosome undergoing rearrangement, but there is certainly one less chromosome pair in the latter. This is the primitive condition proposed for members of the *Orlitia* complex, a karyotype retained by *Orlitia.* Another 2n=50 lineage relocated the NOR interstitially on the long arm of a large microchromosome pair. This is characteristic of the batagurine genus *Malayemys* and the emydines. The common possession of a derived karyotypic condition in these two separate groups is suggestive of a relationship which may be spurious due to convergence or may be indicative of a close relationship between *Malayemys* and those batagurines which gave rise to emydines (assuming *Malayemys* is not actually an emydine and that emydines arose from some batagurine). Figure 11 schematically represents the phylogenetic relationships among emydid turtles suggested by the latter scenario.

Rhinoclemmys stands as the only New World genus of the Batagurinae, a relationship supported by the karyotypic data (Bickham & Baker, 1976a, b; Killebrew, 1977). The karyotypic data provide no real evidence of its relationships within the batagurines, as the primitive *Rhinoclemmys* karyotype differs only slightly from that of the entire subfamily. The chromosomally divergent *R. p. punctularia* and *R. funerea* would seem to have attained their derived karyotypes from more typical *Rhinoclemmys,* with *R. p. punctularia* being the most highly derived.

McDowell (1964) partitioned the emydids into two subfamilies and proposed several groups of related genera. It appears that all genera in the Emydinae possess identical karyotypes (Bickham & Carr, 1983). The batagurines were grouped into four complexes of related genera by McDowell (1964), and Bramble (1974) subsequently added a

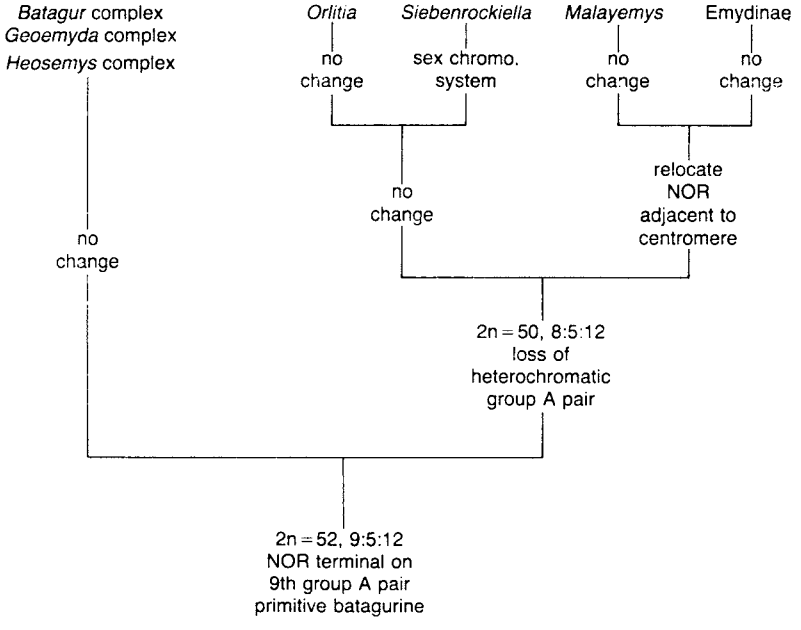

Fig. 11. Schematic representation of the phylogenetic relationships suggested by cladistic analysis of karyotypic data.

fifth (Table 1). All but the *Hardella* complex have been karyotypically examined. Much of the proposed generic complex classification is untestable using karyology because so many batagurines have retained the primitive karyotype. The karyotypic data do support the close relationship between *Orlitia* and *Siebenrockiella* allied in the *Orlitia* complex. The inclusion of *Malayemys* in the *Batagur* complex is not supported by the karyotypic data. Its relationships would seem to lie somewhere between the *Orlitia* complex and the Emydinae (as depicted in Fig. 11). We consider the genus *Malayemys* as constituting its own, distinct generic assemblage, equivalent in rank to the other five previously proposed (Table 3).

Table 3. Suggested classification of generic groups in the Batagurinae.

Hardella complex	(no karyotypic data)
Batagur complex	2n = 52, 9:5:12, NOR on 9th group A pair
Geoemyda complex	2n = 52, 9:5:12, NOR on 9th group A pair
Heosemys complex	2n = 52, 9:5:12, NOR on 9th group A pair
Orlitia complex	2n = 50, 8:5:12, NOR terminal on microchromosome
Malayemys complex	2n = 50, 8:5:12, NOR proximal to centromere on microchromosome

A recent study of biochemical relationships in the Batagurinae sheds some light on intergeneric relationships (Sites et al., 1984). As with karyotypes, the genera *Siebenrockiella* and *Orlitia* are shown to be closely related electrophoretically. *Malayemys,* however, appears most closely related to *Ocadia,* indicating the karyotypic divergence of *Malayemys* may be of relatively recent occurrence.

The generic groupings of McDowell (1964) and Bramble (1974) are not strongly supported by the karyological and electrophoretic data sets (with the exception of the *Orlitia* complex). However, it should be emphasized that karyology is too conservative (and electrophoresis too variable) to be very useful at this level of divergence in these turtles. Further study of batagurine systematics should employ biochemical or morphological features that have evolved at a rate intermediate between karyology and protein electrophoresis.

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References

Albrecht, P. W., 1976. The cranial arteries of turtles and their evolutionary significance. J. Morphol. 149: 159–182.

Barros, R. B., Sampaio, M. M., Assis, M. R., Ayres, M. & Cunha, O., 1975. A karyological study of Geoemyda punctularia punctularia (Daudin, 1802) from the Amazon region of Brazil (Chelonia, Emydidae). Acta Amazon. 5: 95–96.

Bickham, J. W., 1975. A cytosystematic study of turtles in the genera Clemmys, Mauremys, and Sacalia. Herpetologica 31: 198–204.

Bickham, J. W. & Baker, R. J., 1976a. Chromosome homology and evolution of emydid turtles. Chromosoma 54: 201–219.

Bickham, J. W. & Baker, R. J., 1976b. Karyotypes of some neotropical turtles. Copeia 1976: 703–708.

Bickham, J. W. & Baker, R. J., 1979. Canalization model of chromosomal evolution. Bull. Carnegie Mus. Nat. Hist. 13: 70–84.

Bickham, J. W., Bjorndal, K. A., Haiduk, M. W. & Rainey, W. E., 1980. The karyotype and chromosomal banding patterns of the green turtle (Chelonia mydas). Copeia 1980: 540–543.

Bickham, J. W. & Carr, J. L., 1983. Taxonomy and phylogeny of the higher categories of cryptodiran turtles based on a cladistic analysis of chromosomal data. Copeia 1983: 918–932.

Boulenger, G. A., 1889. Catalogue of the Chelonians, Rhynchocephalians, and Crocodiles in the British Museum (Natural History). London: Trustees of the British Museum.

Bramble, D. M., 1974. Emydid shell kinesis: biomechanics and evolution. Copeia 1974: 707–727.

Carr, J. L. & Bickham, J. W., 1981. Sex chromosomes of the Asian black pond turtle, Siebenrockiella crassicollis (Testudines: Emydidae). Cytogenet. Cell Genet. 31: 178–183.

DeSmet, W. H. O., 1978. The chromosomes of 11 species of Chelonia (Reptilia). Acta zool. path. antverpiensia No. 70: 15–34.

Dowler, R. C. & Bickham, J. W., 1982. Chromosomal relationships of the tortoises (family Testudinidae). Genetica 58: 189–197.

Ernst, C. H., 1978. A revision of the neotropical turtle genus Callopsis (Testudines: Emydidae: Batagurinae). Herpetologica 34: 113–134.

Ernst, C. H., 1981. Rhinoclemmys punctularia. Cat. Amer. Amph. Rept. 276: 1–2.

Goodpasture, C. & Bloom, S. E., 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma 53: 37–50.

Haiduk, M. W. & Bickham, J. W., 1982. Chromosomal homologies and evolution of testudinoid turtles with emphasis on the systematic placement of Platysternon. Copeia 1982: 60–66.

Killebrew, F., 1977. Mitotic chromosomes of turtles. IV. The Emydidae. Tex. J. Sci. 29: 245–253.

Killebrew, F. & McKown, R. R., 1978. Mitotic chromosomes of Gopherus berlandieri and Kinixys belliana belliana (Testudines, Testudinidae). Southw. Nat. 23: 162–164.

Levan, A., Fredga, K. & Sandbert, A. A., 1964. Nomenclature for centromeric position on chromosomes. Hereditas 50: 201–220.

Loveridge, A. & Williams, E. E., 1957. Revision of the African tortoises and turtles of the suborder Cryptodira. Bull. Mus. comp. Zool. 115: 163–557.

McDowell, S. B., 1961. On the major arterial canals in the ear-region of testudinoid turtles and the classification of the Testudinoidea. Bull. Mus. Comp. Zool. 125: 23–39.

McDowell, S. B., 1964. Partition of the genus Clemmys and related problems in the taxonomy of the aquatic Testudinidae. Proc. Zool. Soc. London 143: 239–279.

Nakamura, K., 1949. A study in some chelonians with notes on chromosomal formula in the Chelonia. Kromosomo 5: 205–213.

Parsons, T. S., 1968. Variation in the choanal structure of Recent turtles. Can. J. Zool. 46: 1235–1263.

Pritchard, P. C. H., 1979a. Taxonomy, evolution and zoogeography. In Turtles: Perspectives and research (M. Harless and H. Morlock, eds). New York, Wiley, pp. 1–42.

Pritchard, P. C. H., 1979b. Encyclopedia of Turtles. Hong Kong: T. F. H. Publications.

Seabright, M., 1971. A rapid banding technique for human chromosomes. Lancet 1971–II: 971–972.

Singh, L.,1972. Karyological studies of three species of turtles from India. Indian Biologist 4: 64–69.

Sites, J. W., Jr., 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. Syst. Zool. 33: 137–158.

Sites, J. W., Jr., Bickham, J. W., Haiduk, M. W. & Iverson, J. B., 1979. G-banded karyotypes of six taxa of kinosternid turtles. Copeia 1979: 692–698.

Smith, H. M. & James, L. F., 1958. The taxonomic significance of cloacal bursae in turtles. Trans. Kans. Acad. Sci. 61: 86–96.

Stock, A. D., 1972. Karyological relationships in turtles (Reptilia: Chelonia). Can. J. Genet. Cytol. 14: 859–868.

Sumner, A. T., 1972. A simple technique for demonstrating centromeric heterochromatin. Expl Cell Res. 75: 304–506.

Wermuth, H. & Mertens, R., 1977. Liste der rezenten Amphibien und Reptilien. Testudines, Crocodylia, Rhynchocephalia. Das Tierreich 100: xxvii + 174 pp.

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