On the Monophyly of the Agamid Genus *Gonocephalus* Kaup, 1825 (Reptilia: Squamata): A Chromosomal Perspective

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Abstract: We karyotyped five species of the agamid genus *Gonocephalus*, *G. chamaeleontinus*, *G. liogaster*, *G. bellii*, *G. grandis* (from Peninsular Malaysia), and *G. robinsonii*. Of these, karyotypes of the first four species had several chromosomal characteristics exclusively shared with the previously reported karyotypes of *G. miotympanum* and *G. grandis* (from Borneo), such as the diploid chromosome number (42) and the presence of 22 banded macrochromosomes. This seems to support the monophyly of those four species and *G. miotympanum*, probably along with some other species of the genus not yet karyotyped. This hypothesis is premised on our finding of distinct chromosomal characteristics that are indicative of highly derived states in the agamid karyotypes. The karyotype of *G. robinsonii*, while remarkably different from other congeneric karyotypes in exhibiting much smaller diploid (32) and banded macrochromosome numbers (12), share these and other chromosomal characteristics with several Australian species. It seems unlikely for the karyotype of *G. robinsonii* to directly emerge from other congeneric karyotypes or *vice versa*. We conclude that the inclusion of this species in *Gonocephalus* would render the genus paraphyletic.

Key words: Reptilia; Agamidae; *Gonocephalus bellii*; *G. chamaeleontinus*; *G. liogaster*; *G. grandis*; *G. robinsonii*; Karyotype; Monophyly

INTRODUCTION

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The agamid genus *Gonocephalus* Kaup, 1825, is a group of moderate-sized to large lizards. Darlington (1957) argued that the

genus is zoogeographically exceptional, because it was then considered to occur on both sides of Wallace's Line, a zoogeographic border between the Oriental and Australian faunas. Based on the microchromosome numbers, Witten (1983) also postulated that the Australian species assigned to *Gonocephalus* at that date represent recent dispersals from Southeast Asia across Wallace's Line.

In his unpublished Ph.D. dissertation, Moody (1980), on the basis of phylogenetic analysis of morphological characters, asserted that the *Gonocephalus* species east of Wallace's line were derived from radiations of the Australian stock, and that they are phylogenetically distant from the Southeast Asian species. He further argued that the genus *Hypsilurus* Peters, 1867, once synonymized to *Gonocephalus* by Boulenger (1885), should be resurrected to accommodate species from the Australian Region. Results of more recent immunogenetic (Baverstock and Donnellan, 1990; King, 1990), karyological (Ota et al., 1992), electron-microscopic (Ananjeva and Matveyeva-Dujsebayaeva, 1996), and molecular studies (Honda et al., 2000) favored Moody's (1980) view. All recent authors, with the exception of a few who have obviously overlooked these works (e.g., Urban, 1999), restrict the application of the generic name, *Gonocephalus*, to the Southeast Asian species (Welch et al., 1990; Manthey and Grossmann, 1997).

In all those works addressing the phylogeny of *Gonocephalus* (sensu lato), however, the species assemblage on the western side of Wallace's Line (i.e., *Gonocephalus* [sensu stricto]), though constituting no less than 16 species (Welch et al., 1990; Manthey and Grossmann, 1997), was represented by very few species. For example, Moody (1980) examined osteological specimens for only five species. Furthermore, Baverstock and Donnellan (1990), Ota et al. (1992), Ananjeva and Matveyeva-Dujsebayaeva (1996), and Honda

et al. (2000) examined only one, two, four, and one species, respectively. Considering that a thorough definition of *Gonocephalus* (sensu stricto) depends only on a few external characters (Moody, 1980; Manthey and Grossmann, 1997), the monophyly of the genus is obviously yet to be examined.

Ota et al. (1992) reported that *G. grandis* and *G. miotympanum*, both from Borneo, share characteristic chromosomal arrangements that are obviously in highly derived states. This suggests that the karyological approach may be an effective way to examine the monophyly of the genus. Therefore, in this study, we karyotyped four additional species of *Gonocephalus* including its type species, *G. chamaeleontinus*, as well as *G. grandis* from the Peninsular Malaysia.

MATERIALS AND METHODS

Except for two male *Gonocephalus robinsonii*, all lizards, collected from Peninsular Malaysia and Pulau Tioman (Table 1), were transported to the laboratory where they were injected intraperitoneally with 0.1 ml of colchicine solution (2 mg/ml) per gram of body weight. Sixteen to 18 h after injection, they were anesthetized with diethyl ether and were dissected to remove femur bones. Bone marrow were flushed out from the bones with Hanks balanced buffer solution. For each sample, the cell suspension was left to stand for 10 min before it was centrifuged at 2000 rpm for 5 min. Bone marrow cells were then treated with hypotonic KCl solution (0.06 mole/l) at room temperature (26–28°C) for 1 h, followed by fixation in a 1 : 3 glacial acetic acid : absolute methyl alcohol mixture. Mitotic chromosome slides were prepared by the splash technique, air-dried, and were stained in 6% Gurr Giemsa (BDH) solution. Mitotic cell slides for the remaining two male *G. robinsonii* were prepared in the field following Ota (1989a), and were stained in 2% Giemsa solution.

TABLE 1. Localities, sizes, and sexual compositions of samples of five *Gonocephalus* species examined in this study.

Species	N			Locality
	Males	Females	Total	
<i>G. bellii</i>	2	3	5	Gombak Forest Reserve, Peninsular Malaysia (03°09' N, 101°39' E)
<i>G. chamaeleontinus</i>	3	0	3	Pulau Tioman, near Peninsular Malaysia (02°49' N, 104°09' E)
<i>G. liogaster</i>	0	3	3	Gombak Forest Reserve, Peninsular Malaysia (03°09' N, 101°39' E)
<i>G. grandis</i>	3	3	6	Pulau Tioman, near Peninsular Malaysia (02°49' N, 104°09' E)
<i>G. robinsonii</i>	3	0	3	Cameron Highlands Peninsular Malaysia (04°28' N, 101°20' E)

Karyotypes were determined for each individual lizard on the basis of 8–20 well-spread metaphase cells. Selected cell spreads were photographed with a Nikon Optiphot 2 Photomicrography camera using Kodak TMAX ASA 100 film. Individual chromosome pairs were arranged in decreasing size. For the calculation of arm ratio for each chromosome pair, the lengths of chromosome arms were measured with a CALCOM digitizer. Terminology for chromosomal descriptions follows Green and Sessions (1991), and the karyotype formula follows Peccinini-Seale (1981). Voucher specimens were deposited in the Zoological Reference Collection, Depart-

ment of Biological Sciences, National University of Singapore (ZRC) and Herpetological Collection, Department of Zoology, Kyoto University (KUZ).

RESULTS

In all *Gonocephalus* species examined, karyotypes consisted of chromosomes forming large and smaller size-groups that are referred to here as macrochromosomes and microchromosomes, respectively (Table 2). Of these, macrochromosomes were all bi-armed, whereas detailed morphology remained undetermined for most microchromosomes. No sex chromosome hetero-

TABLE 2. Karyotypes of species of the genus *Gonocephalus*. M=macrochromosomes; m=microchromosomes.

Species	2n	Arm nos. in macro- chromosomes	Chromosomal formula	Source
<i>G. bellii</i>	42	44	22 M + 20 m	this study
<i>G. chamaeleontinus</i>	42	44	22 M + 20 m	this study
<i>G. liogaster</i>	42	44	22 M + 20 m	this study
<i>G. robinsonii</i>	32	24	12 M + 20 m	this study
<i>G. grandis</i> (Pulau Tioman)	42	44	22 M + 20 m	this study
<i>G. grandis</i> (Borneo)	42	44	22 M + 20 m	Ota et al. (1992)
<i>G. miotympanum</i>	42	44	22 M + 20 m	Ota et al. (1992)

morphisms or secondary constrictions were evident in any karyotypes.

Karyotypes of *G. chamaeleontinus*, *G. liogaster*, *G. bellii*, and *G. grandis* consisted of $2n=42$ chromosomes, including 22 macrochromosomes (pairs 1–11) and 20 microchromosomes (pairs 12–21). The macrochromosomes of *G. chamaeleontinus* and *G. liogaster* were all metacentric (Fig. 1). From these, macrochromosomes of *G. bellii* and *G. grandis* differed in including submetacentric elements in pairs 1, 4 and 10, and pairs 2, 5, 7 and 9, respectively (Fig. 2). Thus, the arm numbers in macrochromosomes were 44 in all of the four karyotypes. With respect to the microchromosomes, the largest pair (i.e., pair 12) of the *G. bellii* karyotype was distinctly enlarged compared to the chromosome pair immediately following, thus obscuring the size-gap difference between the macro- and microchromosomes. In contrast, chromosome pair 12 was almost as small as pair 13 in karyotypes of *G. chamaeleontinus*, *G. liogaster*, and *G. grandis*, resulting in a more prominent size-gap difference between the two groups of chromosomes.

The karyotype of *G. robinsonii* differs remarkably from those of the other four *Gonocephalus* species in having substantially fewer ($2n=32$) chromosomes. Of the diploid chromosomes, 12 (pairs 1–6) were metacentric macrochromosomes, whereas the remaining 20 (pairs 7–16) were microchromosomes (Fig. 3). Therefore, the arm number in macrochromosomes of this karyotype equaled 24.

DISCUSSION

Karyotypes of *G. chamaeleontinus*, *G. bellii*, *G. liogaster*, and *G. grandis* from Peninsular Malaysia and Pulau Tioman share similar chromosomal features with those of *G. miotympanum* and *G. grandis* from Borneo (Ota et al., 1992). In contrast, the karyotype of *G. robinsonii* differs strikingly from other congeneric karyo-

types.

In the family Agamidae, two karyomorphs are typical: (1) $2n=34$ or 36 chromosomes, including six pairs of metacentric or submetacentric macrochromosomes and 11 or 12 pairs of microchromosomes; and (2) $2n=46$ or 48 chromosomes, all telocentric chromosomes without a distinct size-break (Bickham, 1984; King, 1981; Moody and Hutterer, 1978; Olmo, 1986; Ota and Hikida, 1989; Solleder and Schmid, 1988; Witten, 1983). One of these karyomorphs is considered to be derived from the other through a series of Robertsonian rearrangements of macrochromosomes, sometimes accompanied with addition or deletion of one microchromosome pair (Bickham, 1984; King, 1981). Judging from the fact that both of the two karyomorphs sometimes occur in a single genus or closely related genera (Gorman and Shochat, 1972; Ota, 1988, 1989b) and that there are so few karyotypes representing intermediate states between the two extremes, such chromosomal rearrangements may proceed rapidly when they are once triggered (King, 1981).

It is obvious that the karyotypes of *G. chamaeleontinus*, *G. bellii*, *G. liogaster*, *G. grandis*, and *G. miotympanum* could not be derived from either of the two typical agamid karyomorphs merely through Robertsonian rearrangements of macrochromosomes and slight numerical changes of microchromosomes, because these karyotypes include a much larger number of biarmed macrochromosomes (22) than those of any other agamid karyotypes hitherto reported, and at the same time, exhibit a greater diploid number (42) than the agamid karyomorph (1). Moreover, their fundamental number (NF, >64) is considerably greater than those with karyomorph (2) (NF=46 or 48). We thus consider karyotypes of the five *Gonocephalus* species to represent a highly derived state, and that the chromosomal features exclusively shared among these species support

FIG. 1. Karyotypes of (A) *Gonocephalus chamaeleontinus*, and (B) *G. liogaster*. Bars equal 5 μ m.

FIG. 2. Karyotypes of (A) *Gonocephalus bellii*, and (B) *G. grandis*. Bars equal 5 μm .

FIG. 3. Karyotype of *Gonocephalus robinsonii*. Bar equals 5 μ m.

their monophyly, presumably along with some other species of the genus not yet studied karyologically.

The karyotype of *G. robinsonii* is similar to karyomorph (1), and considering remarkable differences in the diploid number of chromosomes or the arm number of macrochromosomes between this and other congeneric karyotypes (Table 2), it is unlikely that the karyotype of *G. robinsonii* directly arose from other congeneric karyotypes or *vice versa*. Thus, we conclude that the inclusion of this species in *Gonocephalus* would render the genus paraphyletic.

The number of microchromosomes (20) in the *G. robinsonii* karyotype is smaller than that in the typical agamid karyomorph (1)(22 or 24; Bickham, 1984; King, 1981). Such a chromosomal arrangement (i.e., 12M+20m) is exclusively shared with several agamid species that are supposedly derived from the Australian endemic radiation (Witten, 1983). It is thus probable that

G. robinsonii actually represents dispersals from the Australian Region into Southeast Asia like *Physignathus cocincinus* (see Honda et al., 2000). However, it is also probable that the karyotype of *G. robinsonii* is derived from karyomorph (1), exhibited by a number of other agamid species including several from Southeast Asia (see Ota and Hikida [1989]), through deletions of one or two microchromosome pairs. More comprehensive analyses using biochemical and molecular approaches are needed to determine the relationship of this enigmatic species with certainty.

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