# Molecular Evaluation of Phylogenetic Significances in the Highly Divergent Karyotypes of the Genus *Gonocephalus* (Reptilia: Agamidae) from Tropical Asia

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**ABSTRACT**—The Oriental large-bodied crested dragons of the genus *Gonocephalus* are known to include two distinct karyomorphs. To evaluate their phylogenetic significances, we conducted phylogenetic analyses of the genus together with other agamid genera on the basis of 862 base positions of mitochondrial 12S and 16S rRNA genes. Results suggested the presence of two distinct lineages within *Gonocephalus*, of which one, represented by *G. robinsonii* that has a 2n=32 karyotype, was closer to other Oriental agamid genera than to the other congeneric lineage. Monophyly of the latter, characterized by unique chromosomal arrangement among agamid genera (2n=42 karyotype), was confirmed. It is thus likely that states of morphological characters shared between the two lineages are derived through convergence, or represent symplesiomorphy. Our results also suggest that the karyological similarity between *G. robinsonii* and several Australian agamids, pointed out in a previous study, is actually attributable to homoplasy rather than their recent common ancestry.

**Key Words**: Agamidae, *Gonocephalus*, mitochondrial DNA, phylogeny, biogeography

## INTRODUCTION

The agamid genus *Gonocephalus* Kaup, 1825, originally considered as consisting of 29 species distributed in Southeast Asia, Australia, New Guinea and the Solomons (Wermuth, 1967), was highlighted as a zoogeographical exception because of its occurrence on both sides of the Wallacia, a border of the Oriental and Australian faunal realms (e.g., Darlington, 1957). In his unpublished dissertation, Moody (1980), on the basis of cladistic analyses using morphological characters, argued that species of the genus *Gonocephalus* from Australia, New Guinea and the Solomons were not actually closely related to the Oriental congeners. He thus re-assigned the former to the resurrected genus *Hypsilurus* Peters, 1867. Such an arrangement, published by Welch *et al.* (1990), was favored by recent immunological (Baverstock and Donnellan, 1990; King, 1990), karyological (Ota *et al.*, 1992), electron-micro-

scopic (Ananjeva and Matveyeva-Dujsebayava, 1996), and molecular studies (Honda *et al.*, 2000; Macey *et al.*, 2000).

Recent karyological studies further revealed prominent chromosomal variation within *Gonocephalus* sensu stricto: four of the five species examined shared a 2n = 42 karyotype including 22 biarmed macrochromosomes, whereas the remainder, *G. robinsonii*, had a 2n = 32 karyotype including no more than 12 macrochromosomes (Ota *et al.*, 1992; Diong *et al.*, 2000). Based on the fact that the former arrangement is exceptional as of the agamid karyotype, Diong *et al.* (2000) interpreted it as a synapomorph of *Gonocephalus* exclusive of *G. robinsonii*, and went so far as to argue for the possible paraphyletic nature of the genus. They, on the basis of similarities in the number of microchromosomes between *G. robinsonii* and a few Australian agamids, also noted that *G. robinsonii* may be an Asiatic representative of the Australian agamid radiation, like *Physignathus cocincinus* (Honda *et al.*, 2000; Macey *et al.*, 2000). However, these hypotheses seriously suffer due to the lack of appropriate phylogenetic analysis of chromosome characters and of comparable karyological data for

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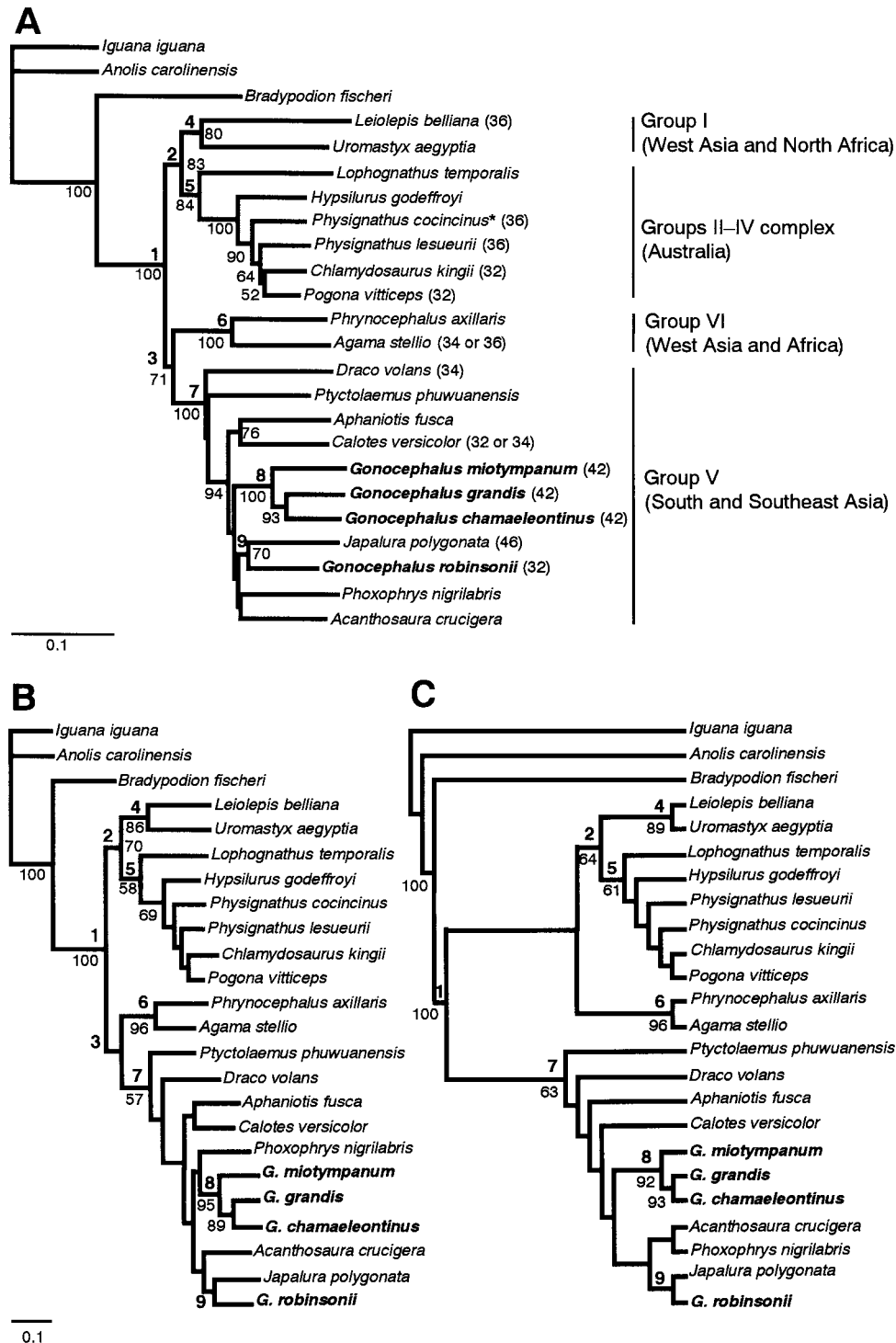    E-mail: g019002@lab.u-ryukyu.ac.jp

Fig. 1. (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 1,000 bootstrap replications. Nodes with bold numbers indicate relationships referred to in the text. Bar equals 0.1 unit of Kimura's two-parameter distance. Published data for the diploid chromosome numbers (2n) are given in parentheses (DeSmet, 1981; Witten, 1983; Solleder and Schmid, 1988; Ota *et al.*, 1992; Ota and Hikida, 1989; Diong *et al.*, 2000; Ota, unpubl data). Although Groups II–IV complex is mainly distributed in Australasia, *Physignathus cocincinus* is occurs in the continental part of Southeast Asia. (B) Maximum-likelihood (ML) dendrogram (ln likelihood=–11727.6). 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 option (one parsimonious tree, 2,418 steps, 448 bp informative under the condition of parsimony, consistency index=0.45, homoplasy index=0.55, retention index=0.48). Branches without BP values were not supported in≥50% of the 1,000 bootstrap replicates.

many other related genera.

In the present study, we examined the phylogenetic relationships of the two karyomorphs of *Gonocephalus* and other agamid genera by analyzing mitochondrial DNA sequence data. Our purposes are to examine consistency between patterns in chromosomal and phyletic diversification within the *Gonocephalus*, and to test the closer affinity of *G. robinsonii* to the Australian agamids as hypothesized by Diong *et al.* (2000).

## MATERIALS AND METHODS

Three species of the *Gonocephalus* sensu stricto, *G. chamaeleontinus* from West Malaysia and Indonesia, *G. miotympanum* from Borneo and *G. robinsonii* from West Malaysia (Welch *et al.*, 1990; Manthey and Grossmann, 1997), were newly examined in the present study (see Appendix for locality data of specimens examined). We incorporated published data representing the six major agamid groups of Moody (1980) (Groups I–VI), the Chamaeleonidae (*Bradypodion fischeri*), the Polychridae (*Anolis carolinensis*), and the Iguanidae (*Iguana iguana*) (see Appendix for accession numbers). Of these, the last three species were regarded as outgroups, because these families are considered to be basal to the Agamidae (Frost and Etheridge, 1989; Macey *et al.*, 1997; Honda *et al.*, 2000)

Extraction, amplification and sequencing of DNA are described in detail elsewhere (Honda *et al.*, 1999a, b). A part of mitochondrial 12S and 16S rRNA genes consisting of approximately 860 base pairs (bp) 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 unambiguously determined based on maximum nucleotide similarity following Honda *et al.* (2000).

The neighbor-joining (NJ) method (Saitou and Nei, 1987) was applied to infer relationships among taxa on the basis of a pairwise matrix of the distance from Kimura's (1980) two-parameter model, using CLUSTAL X 1.8 (Thompson *et al.*, 1994). The maximum-likelihood (ML, empirical base frequencies and equal rate substitution model) and maximum-parsimony (MP, no bias between transition and transversion) analyses were also conducted using heuristic search option of PAUP* 4.0b (Swofford, 1998). In these three analyses, gap sites were excluded, and confidences of branched were assessed by bootstrap resamplings (Felsenstein, 1985).

## RESULTS

The amplified fragment of the 12S and 16S rRNA genes consisted of 862 total sites. Of these, however, 201 sites involved in the insertion or deletion were excluded from phylogenetic analyses. The NJ dendrogram derived from aligned sequences is shown in Fig. 1A. The monophyly of the Agamidae was supported in all bootstrap iterations (node 1: 100%). The ingroup portion of this dendrogram was divided into two major lineages. One of the major clusters (node 2: 83%) further split into two subclusters (nodes 4, 5), of which node 4 (80%) consisted of West Asian and North African primitive agamids (*Leiolepis* and *Uromastyx*: Group I sensu Moody, 1980), whereas node 5 (84%) accommodated Australian genera including *Hypsilurus* that had been previously assigned to *Gonocephalus* (Groups II–IV complex). The other major cluster (node 3: 71%) split into two

subclusters (nodes 6, 7), of which node 6 (100%) consisted of West Asian and African derived agamids (*Agama* and *Phrynocephalus*: Group VI). Node 7 (100%), on the other hand, contained Southeast Asian derived agamids (*Acanthosaura*, *Aphaniotis*, *Calotes*, *Draco*, *Gonocephalus*, *Japalura*, *Phoxophrys* and *Ptyctolaemus*: Group V). Within this node, *G. chamaeleontinus*, *G. grandis* and *G. miotympanum* (node 8: 100%), and *G. robinsonii* and *J. polygonata* (node 9: 70%) constituted exclusive clusters, respectively.

Relationships depicted as a result of ML (Fig. 1B) and MP analyses (Fig. 1C) showed no inconsistency with those expressed in the NJ dendrogram in terms of topology of nodes 1–9, except for the absence of node 3 in MP. However, when the Kishino and Hasegawa (1989) test and Templeton (1983) test were applied to ML and MP dendrograms, respectively, topologies in Fig. 1B and Fig. 1C were not significantly different from those in alternative hypotheses in which *Gonocephalus* was constrained monophyletic (P=0.130 and 0.167, respectively).

## DISCUSSION

Several authors pointed out that there are two typical karyomorphs in the family Agamidae, of which one consists of 2n=34 or 36 chromosomes including 12 biarmed macrochromosomes and 22 or 24 microchromosomes, whereas the other of 2n=46 or 48, 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 by addition or deletion of a pair of microchromosomes (Bickham, 1984; King, 1981). Based on these empirical assumptions, Ota *et al.* (1992) and Diong *et al.* (2000) hypothesized that the 2n=42 karyomorph with 22 biarmed macrochromosomes and 20 microchromosome of *Gonocephalus bellii*, *G. chamaeleontinus*, *G. grandis*, *G. liogaster* and *G. miotympanum* represents a highly specialized state. The latter authors also considered such an arrangement to be a strong evidence for the monophyly of those species against *G. robinsonii*, a species having 2n=32 karyotype including only 12 biarmed macrochromosomes, and further assumed the latter species to be distant from other congeners phylogenetically.

Present results, consistently indicating the monophyly of the "2n=42 species" of *Gonocephalus* against *G. robinsonii* and other related genera with high bootstrap values, offer a substantial support to these hypotheses, although the number of species examined in the present study is too small to draw any definite conclusion. The states of morphological characters shared between *G. robinsonii* and other congeners (i.e., diagnostic characters of *Gonocephalus*), such as the presence of tubercular scales among otherwise homologous granular scales (Manthey and Grossmann, 1997), thus appear to be derived from convergence or rep-

resent symplesiomorphy.

As was pointed out by Diong *et al.* (2000), chromosomal arrangement of *G. robinsonii* most resembles that of a few Australian agamids in that it has only 20 microchromosomes besides 12 biarmed macrochromosomes. Present results, however, negate their close affinity, and indicate the origin of *G. robinsonii* in an Asiatic agamid radiation. Simple deletion of a pair of microchromosomes seems to be responsible for the emergence of karyotype of *G. robinsonii*.

## ACKNOWLEDGMENTS

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**APPENDIX.** DDBJ accession numbers of mitochondrial 12S and 16S rRNA gene sequences used in this study (given in parentheses). Localities and catalogue numbers are also given for specimens newly sequenced this time. These, as well as previously used specimens (Honda *et al.*, 2000), were deposited in the Herpetological Collection of Department of Zoology, Kyoto University (KUZ).

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