Karyotypes of Five Hylid Frogs from Papua New Guinea, with a Discussion on Their Systematic Implications

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Abstract: The karyotypes of three species of the genus Litoria and two of the genus Nyctimystes (Hylidae: Pelodryadinae) from Papua New Guinea are reported. They have \(2n=26\) chromosomes which cannot be divided into distinct size groups. In Litoria species, five or six pairs among pair Nos. 2–8 are submetacentric or subtelocentric and the others metacentric. The karyotype of Nyctimystes differs from that of Litoria in having a longer No. 1 and metacentric No. 2. Karyological comparisons with related taxa suggest that the Pelodryadinae is most closely related to the Phyllomedusinae, and that the primitive leptodactylids, rather than the Australian myobatrachids, are likely to be the ancestral stock of the Pelodryadinae. Nyctimystes seems to have been derived from a group of Litoria.

Key words: Karyotype; Anura; Hylidae; Litoria; Nyctimystes; Papua New Guinea

All hylid frogs of the Australopapuan region are generally assigned to the subfamily Pelodryadinae (Duellman and Trueb, 1986; Frost, 1985) or, by some authors, to a distinct family Pelodryadidae (Laurent, 1979; Savage, 1973). Of the three genera composing this group, Cyclorana is confined to Australia, and Nyctimystes is mostly Papuan in distribution.

Duellman (1967) first reported chromosome numbers of four Litoria and four Nyctimystes species from Papua New Guinea, seven of which had \(2n=26\) and L. angiana had \(2n=30\) chromosomes. Menzies and Tippett (1976), examining 22 Litoria and six Nyctimystes species of Papua New Guinea, reported that the chromosome number of Papuan hylids (including L. angiana) is \(2n=26\), with the exception of L. infrafrenata with \(2n=24\) chromosomes. Karyological studies on the Australian species including the genus Cyclorana (King, 1980; King et al., 1979; Morescalchi, 1979; Morescalchi and Ingram, 1974; Stephenson and Stephenson, 1970) confirmed that the basic diploid number of the pelodryadine frogs is 26.

Excepting the karyotype of L. infrafrenata given by Menzies and Tippett (1976), no detailed karyotypes have been given for Papuan hylid frogs. In this paper, we describe the karyotypes of three Litoria and two Nyctimystes species and compare them with those of the other hylid subfamilies and the related families. The chromosome number of Nyctimystes pulchra is here reported for the first time.

**MATERIALS AND METHODS**

Litoria arfakiana (3 males), L. thesaurensis (1 male), L. wollastoni (4 males), Nyctimystes foricula (2 males), and N. pulchra (1 male) were collected in the vicinity of Wau, Morobe, Papua New Guinea in April 1982. Chromosome spreads were prepared from bone marrow cells by the method of Omura (1967). Chromosomes were measured with a digitizer (Graphtec KD4300) on enlarged photomicrographs. The lengths of secondary contractions were included in the measurements. Chromosome pairs were numbered in the order of relative length. Nomenclature of centromeric position follows Levan et al. (1964). In comparisons of chromosome measurements, differences among means were tested by Student’s t-test.

**RESULTS**

All of the five species examined had \(2n=26\) chromosomes which were not readily classified into distinct size groups (Fig. 1). Relative lengths and arm ratios of chromosomes are given in Table 1.

In the three Litoria species, pair No. 1 is metacentric and five or six pairs among Nos. 2 to 8 were submetacentric or subtelocentric (Table 1). Nos. 9 to 13 were metacentric. Statistical tests revealed that five chromosome pairs did not

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| Pair No. | \(L.\) arfakiana  
(N=20) | \(L.\) thesaurensis  
(N=18) | \(L.\) wollastonii  
(N=24) | \(N.\) foricula  
(N=14) | \(N.\) pulchra  
(N=10) |
<table>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Relative length</td>
<td>Arm ratio</td>
<td>Relative length</td>
<td>Arm ratio</td>
<td>Relative length</td>
</tr>
<tr>
<td>1</td>
<td>12.8 ± 0.13</td>
<td>1.66 ± 0.03 m</td>
<td>12.6 ± 0.13</td>
<td>1.53 ± 0.02 m</td>
<td>12.3 ± 0.10</td>
</tr>
<tr>
<td>2</td>
<td>11.2 ± 0.12</td>
<td>1.90 ± 0.05 sm</td>
<td>10.9 ± 0.13</td>
<td>1.77 ± 0.02 sm</td>
<td>12.2 ± 0.18</td>
</tr>
<tr>
<td>3</td>
<td>11.1 ± 0.12</td>
<td>3.14 ± 0.10 st</td>
<td>10.6 ± 0.14</td>
<td>2.67 ± 0.09 sm</td>
<td>10.5 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>10.1 ± 0.12</td>
<td>1.55 ± 0.04 m</td>
<td>9.8 ± 0.08</td>
<td>1.33 ± 0.02 m</td>
<td>10.1 ± 0.10</td>
</tr>
<tr>
<td>5</td>
<td>9.9 ± 0.14</td>
<td>2.97 ± 0.10 sm</td>
<td>8.1 ± 0.09</td>
<td>2.53 ± 0.07 sm</td>
<td>9.4 ± 0.07</td>
</tr>
<tr>
<td>6</td>
<td>7.9 ± 0.11</td>
<td>1.89 ± 0.05 sm</td>
<td>7.9 ± 0.07</td>
<td>1.98 ± 0.03 sm</td>
<td>7.9 ± 0.09</td>
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<tr>
<td>7</td>
<td>6.4 ± 0.09</td>
<td>2.27 ± 0.05 sm</td>
<td>7.2 ± 0.08</td>
<td>1.73 ± 0.04 sm</td>
<td>7.0 ± 0.08</td>
</tr>
<tr>
<td>8</td>
<td>6.2 ± 0.07</td>
<td>1.60 ± 0.05 m</td>
<td>6.6 ± 0.07</td>
<td>1.97 ± 0.04 sm</td>
<td>6.1 ± 0.07</td>
</tr>
<tr>
<td>9</td>
<td>5.8 ± 0.06</td>
<td>1.44 ± 0.04 m</td>
<td>6.2 ± 0.08</td>
<td>1.67 ± 0.04 m</td>
<td>5.7 ± 0.06</td>
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<tr>
<td>10</td>
<td>5.5 ± 0.05</td>
<td>1.36 ± 0.03 m</td>
<td>5.6 ± 0.06</td>
<td>1.64 ± 0.04 m</td>
<td>5.4 ± 0.07</td>
</tr>
<tr>
<td>11</td>
<td>4.9 ± 0.06</td>
<td>1.33 ± 0.02 m</td>
<td>5.5 ± 0.08</td>
<td>1.48 ± 0.04 m</td>
<td>4.9 ± 0.09</td>
</tr>
<tr>
<td>12</td>
<td>4.3 ± 0.08</td>
<td>1.31 ± 0.02 m</td>
<td>4.7 ± 0.09</td>
<td>1.48 ± 0.03 m</td>
<td>4.5 ± 0.08</td>
</tr>
<tr>
<td>13</td>
<td>3.9 ± 0.08</td>
<td>1.28 ± 0.02 m</td>
<td>4.3 ± 0.07</td>
<td>1.30 ± 0.02 m</td>
<td>4.1 ± 0.09</td>
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FIG. 1. Karyotypes of L. arfakiana (A), L. thesaurensis (B), L. wollastoni (C), N. foricula (D), and N. pulchra (E). Scale equals 10 μm.

differ in either relative length or arm ratio (p > 0.05) between L. arfakiana and L. thesaurensis and seven pairs between L. arfakiana and L. wollastoni and between L. thesaurensis and L. wollastoni. In some chromosome spreads of L. arfakiana, a satellite was observed at the end of the long arm of pair No. 12 (Fig. 1A). Because the lengths of the heterochromatic parts were variable, measurements were made excluding these parts. Litoria thesaurensis had a distinct secondary constriction at the middle of the long arm of pair No. 7 (Fig. 1B).

In the two Nyctimystes species, pair Nos. 1 and 2 were metacentric and Nos. 3 to 8 were submetacentric or subtelocentric except metacentric No. 4 of N. foricula. Numbers 9 to 13 were metacentric except the submetacentric Nos. 9 and 11 of N. foricula. Five pairs of N. foricula and N. pulchra did not differ significantly (p > 0.05) in either relative length or arm ratio. A part of the short arm of No. 4, adjacent to the centromere, was heterochromatic in N. foricula (Fig. 1D).

The numbers of chromosome pairs which did not differ significantly in both relative length and arm ratio between the two genera ranged from 2 to 5 (x = 3.33, 6 combinations), and obviously fewer than the numbers within the genus Litoria (x = 6.3, 3 combinations) and Nyctimystes (5, 1 combination). Remarkable differences between karyotypes of the two genera are that the pair No. 1 is distinctly longer in Nyctimystes than in Litoria and that pair No. 2 is metacentric in Nyctimystes, but submetacentric or subtelocentric in Litoria.

DISCUSSION

Although only one species, L. infrarbreata, has been karyotyped for New Guinean pelodriadine hylids, the karyotypes of about two dozen Australian species (Litoria and Cyclorana) are available for comparison (King, 1980; King et al., 1979; Morescalchi and Ingram, 1974; Stephenson and Stephenson, 1970; Tyler et al., 1978). The Australian and New Guinean species of the genus Litoria have the following karyotypic features in common. (1) All have a diploid number of 26, except L. infrarbreata with 24 chromosomes. (2) Relative lengths decrease gradually without forming distinct size groups. (3) Pair Nos. 1 and 4 (Nos. 1 and 5 in L. wollastoni) are metacentric, the majority of Nos. 2, 3, 5 to 7 submetacentric or subtelocentric, Nos. 8 to 10 either metacentric or submetacentric, and most of Nos. 11 to 13 metacentric. Some Australian Litoria species have a heterochromatic portion or a secondary constriction which is very similar in position to those of L. arfakiana and L. thesaurensis. Although the karyotype of Nyctimystes has the
same characteristics as that of *Litoria*, pair No. 1 in the former is apparently longer than the corresponding pairs in karyotypes of the genera *Litoria* and *Cyclorana*. Also, No. 2, instead of No. 4, is metacentric in karyotypes of *Nectimysetes* species. Based on skeletal characteristics, Tyler and Davies (1979) suggested that the genus *Nectimysetes* has originated from a group of *Litoria*. The karyotype of *Nectimysetes* may have been derived from that of *Litoria*.

There are two major problems as to the relationships of Australopapuan hylids; relationships with the other subfamilies of the Hylidae and those with their presumed ancestral stock, Leptodactylidae (see Savage, 1973; Zweifel and Tyler, 1982). So far as the karyological evidence is concerned, phylomedusine hylids examined (three *Agalychnis*, one *Pachymedusa*, and eight *Phyllomedusa* species excepting tetraploid population of *P. burmeisteri*) have 2n=26 chromosomes as pelodryadine hylids (Duellman and Cole, 1965; Barrio, 1976; see Kuramoto, 1990 for further reference). No numerical data for chromosome size and shape are available for the phylomedusine karyotypes. The photographic representations of karyotypes of seven phylomedusine species, however, clearly indicate that their karyotypes are very similar to those of the pelodryadine hylids; distinct size groups are not recognized, pair Nos. 1 and 4 are metacentric, and many pairs are submetacentric or subtelocentric. Although the two subfamilies are separable by several morphological features (Tyler and Davies, 1978), close karyological similarities seem to indicate their close phylogenetic relationships. It is highly probable that the karyotype of pelodryadine and phylomedusine hylids is primitive and conservative (Bogart, 1973).

Of the 12 examined species of another hylid subfamily, the Hemiphractinae (five genera), eight (one *Cryptobatrachus*, one *Fritziana*, five *Gastrotheca*, and one *Stefania* species) also have 2n=26 chromosomes (Bogart, 1973; Schmid et al., 1988; see Kuramoto, 1990 for further reference). The karyotype of the 26-chromosome hemiphractine species consists of five large and eight small chromosome pairs, and some of the small pairs are telocentric. Thus, the karyotype of Hemiphractinae differs from those of Pelodryadinae and Phylomedusinae. None of about 110 examined species of the subfamily Hylinae (14 genera) has 26 chromosomes (Bogart, 1973; Duellman and Cole, 1965; see Kuramoto, 1990 for further reference). The karyotype of Pelodryadinae differs remarkably from that of Hylinae.

It is generally accepted that the family Hylidae is derived from Leptodactylidae (Bogart, 1973). The latter contains four subfamilies, Ceratophryinae, Hylodinae, Leptodactylinae, and Telmatobinae (Frost, 1985). According to Lynch (1971), Ceratophryinae and Telmatobinae are more primitive than Hylodinae (his Elosiinae) and Leptodactylinae. He has discussed in some detail the subfamilial relationships with respect to chromosome numbers and karyotypes (Lynch, 1971, 1978). Compiling the karyological data published thereafter (Barrio and Rinaldi de Chieri, 1971; Bogart, 1970; see Kuramoto, 1990 for further reference), the following generalizations become evident; (1) All examined species of Ceratophryinae (two genera, eight species) and Hylodinae (two genera, eight species) have 2n=26 chromosomes excepting octaploid populations. (2) In Telmatobinae, 10 genera (*Atelognathus*, *Cycloramphus*, *Telmatobius*, etc., 25 species) have 2n=26, three genera (*Alidoses*, *Eleutherodactylus*, and *Syrrophus*) contain species with 2n=26 (19 species) and other diploid numbers (60 species), and 11 genera (*Euphophus*, *Odontophrynus*, etc., 19 species) have diploid numbers other than 26. (3) In Leptodactylinae only four species belonging to the genera *Adenomera* and *Limnomeda* have 2n=26, and 52 other examined species (nine genera) have diploid numbers less than 26, mostly 2n=22. Apparently the number of chromosomes tends to decrease from a primitive 2n=26 to an advanced 2n=22. The karyotypes of Ceratophryinae and Hylodinae consist of five large and eight small chromosomes, whereas those of the 26-chromosome Telmatobinae are composed of chromosome pairs gradually decreasing in size. It is clear that the karyotype of the Pelodryadinae is more similar to those of some members of Telmatobinae than to the species belonging to Ceratophryinae and Hylodinae.

The Australian family Myobatrachidae was separated from the Leptodactylidae rather recently (Savage, 1973). The taxonomic history of this family and the fact that the genus *Cyclorana*, previously included in the Myobatrachidae, is now transferred to the Hylidae imply close relationships between Leptodactylidae, Myobatrachidae, and pelodryadine hylids. Chromosomes of 83 myobatrachid frogs have been reported (Mahony and Robinson, 1986; Morescalchi and Ingram, 1974; see Kuramoto, 1990 for further reference). They have 2n=24 chromosomes.
with the exception of three *Lechriodus* and four *Limnodynastes* species with 2n=22 and two tetraploid *Neobatrachus* with 2n=48 chromosomes. Thus, so far as the karyological data are concerned, the Pelodryadinae seems to be related to the primitive Leptodactylidae rather than to the Myobatrachidae.

**LITERATURE CITED**


要旨 パプアニューギニアのアマガエル5種の
核型とその分類学上の意義

倉本 満・アレン アリソン
パプアニューギニアの Litoria 属3種、Nyc-
timystes 属2種（アマガエル科、ベロドリアス
亜科）のアマガエルの染色体数は2n=26で、
大形・小形染色体の区別は明瞭でない。
Litoria 属に比べて Nytimystes 属の第1染色体
は長く、第2染色体の腕比は小さかった。ベロ
ドリアス亜科の核型は南米のフィロメスサ亜科
のアマガエルに類似し、オーストラリア区のミ
オバトラクス科よりは南米のレプトダクチルス
科の原始的なグループの核型に近い。Nyt-
timystes 属は Litoria 属から派生したものと考えられる。

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