Karyological Analysis of the Rough-Skinned Floating Frog, *Occidozyga lima* (Anura, Dicroglossidae)

Sonsrin, K.[1], & Pinthong, K.[2]*

บทคัดย่อ

การวิเคราะห์แคริโอไทป์ของเขียดจะนา (*Occidozyga lima*) จากภาคตะวันออกเฉียงเหนือของประเทศไทย เตรียมโครโมโซมด้วยวิธีการบดขยี้เซลล์จากเซลล์ไขกระดูกและอัณฑะ ย้อมสีโครโมโซมด้วยเทคนิคการย้อมสีแบบธรรมดา และแถบสีแบบนอร์

ผลการศึกษาพบว่าเขียดจะนามีจำนวนโครโมโซมดิพลอยด์เท่ากับ 26 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 52 ทั้งในเพศผู้และเพศเมีย โครโมโซมประกอบด้วยชนิดเมทาเซนทริกขนาดใหญ่ 4 แท่ง ซับเมทาเซนทริกขนาดใหญ่ 6 แท่ง เมทาเซนทริกขนาดเล็ก 10 แท่ง และซับเมทาเซนทริกขนาดเล็ก 6 แท่ง ตรวจพบโครโมโซมเครื่องหมายที่มีตำแหน่งนอร์อยู่บริเวณใกล้เซนโทรเมียร์ของแขนข้างยาวโครโมโซมคู่ที่ 10 การแบ่งเซลล์ไมโอซิสพบระยะเมทาเฟส 1 ที่มีการแนบชิดของโครโมโซมคู่เหมือนที่ประกอบด้วย 13 วงแหวนไบวาเลนท์ และพบโครโมโซมหนึ่งชุด เฮปพลอดยีในการแบ่งเซลล์ระยะเมทาเฟส 2 เขียดจะนามีสูตรแคริโอไทป์ ดังนี้

ดิพลอยด์ (26) = $L^{m}_{4} + L^{sm}_{6} + S^{m}_{10} + S^{sm}_{6}$

คำสำคัญ : *Occidozyga lima,* แคริโอไทป์, โครโมโซม

[1] Dr. Department of Basic Science, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin 32000, Thailand

[2] Assistant Professor, Dr. Department of Basic Science, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin 32000, Thailand

* Corresponding Author, Email: k_pinthong@yahoo.com

Abstract

Karyological analysis of the rough-skinned floating frog, *Occidozyga lima* (Gravenhorst, 1829) from Northeast, Thailand was carried out. Frog chromosome preparations were conducted by squash technique from bone marrow and testis. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosome with Giemsa's solution.

The results showed that the number of diploid chromosome was 2*n*=26, while the fundamental number (NF) was 52 in both males and females. The types of chromosomes were 4 large metacentric, 6 large submetacentric, 10 small metacentric, and 6 small submetacentric chromosomes. The region adjacent to the subcentromeric region on the long arms of the chromosome pair 10 showed clearly observable nucleolar organizer regions (NORs). We found that during metaphase I the homologous chromosomes showed synapsis, which can be defined as the 13 ring bivalents and 13 haploid chromosomes at metaphase II as diploid species. The karyotype formula is as follows:

$2n\ (26) = L^{m}_{4} + L^{sm}_{6} + S^{m}_{10} + S^{sm}_{6}$

Keywords: *Occidozyga lima*, Chromosome, Karyotype.

1. Background

The rough-skinned floating frog, *Occidozyga lima* (Gravenhorst, 1829) is a species of frog in the Dicroglossidae family. It is found in Bangladesh, Cambodia, China, Hong Kong, India, Indonesia, Laos, Malaysia, Myanmar, Thailand, and Vietnam. Its natural habitats are tropical or subtropical seasonally wet or flooded lowland rivers, grassland, intermittent freshwater lakes, swamps, freshwater marshes, intermittent freshwater marshes, ponds, irrigated land, seasonally flooded agricultural land, and canals and ditches. These frogs are also found in the pet trade (Van Dijk et al., 2004).

Number and morphology of chromosomes are species specific. Information on

chromosomes has been frequently found immensely useful and occasionally even conclusive in distinguishing between closely related taxa. Comparative chromosomal analyses have quite often proved helpful in tracing phylogenetic relationships (Sing, & Banerjee, 2004). There are two reports on cytogenetic studies of *O. lima* namely, Supaprom, & Baimai (2004), and Donsakul (2009) (Table 1).

Table 1

Review cytogenetic reports of the frog's genus *Occidozyga* from Thailand.

Species	2*n*	Karyotype formula	NF	NOR banded	Reference
O. lima	26	12m+14sm	52	-	Supaprom, & Baimai (2004)
	26	18m+8sm	52	-	Donsakul (2009)
	26	16m+10sm	52	2	Present study
O. martensii	26	14m+12sm	52	-	Supaprom, & Baimai (2004)
	26	18m+6sm+2a	52	2	Donsakul, & Rangsiruji (2005)

Remarks: 2*n* = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, sm = submetacentric, a = acrocentric, and - = not available. (Supaprom, & Baimai, 2004; Donsakul, 2009)

In addition, we confirm the results by compare with previous reports. In this study, this is the first report on its chromosome size, standardized idiogram, karyotype formula, and meiotic cell division. The results obtained can provide increasing cytogenetic information for future studies on taxonomy and evolutionary relationships of these frogs.

2. Materials and methods

The *O. lima* samples were obtained from Northeast Thailand. The frogs, ten males and ten females, were transferred to the laboratory and were kept under standard conditions for three days prior to the experiments (Figure 1). Chromosome preparation was conducted by the squash technique from the bone marrow and testis (Patawang, Tanomtong, Phimphan, Chuaynkern, Chuaynkern, Phaengphairee, & Nithikulworawong, 2014; Sangpakdee,

Phimphan, Tengjaroenkul, Pinthong, Neeratanaphan, & Tanomtong, 2017).

Conventional staining, and Ag-NOR banding techniques were applied to stain the chromosomes (Howell, & Black, 1980; Rooney, 2001). The length of short arms (Ls) and long arms (Ll) of the chromosome were measured and the length of total arm chromosomes (LT, LT=Ls+Ll) was recorded. The relative length (RL) and centromeric index (CI) were estimated. The CI was also computed to classify the types of chromosomes according to Turpin, & Lejeune (1965). All parameters were used in karyotyping and idiograming.

Figure 1 General characteristic of the rough-skinned floating frog (*Occidozyga lima*) from Northeast Thailand, scale bar indicates 1 centimetre.

3. Results and Discussions

The diploid number (2*n*) found in *O. lima* was 26 chromosomes in both males and females (Figure 2 and 3). This is the same chromosome number of the *O. lima* as reported in previous studies (Supaprom, & Baimai, 2004; Donsakul, 2009). In comparison with the genus *Occidozyga* in Thailand, the diploid chromosome number of *O. martensii* is 2*n*=26 (Supaprom, & Baimai, 2004; Donsakul, & Rangsiruji, 2005). The fundamental number (NF) was 52 in both males and females. This NF agrees with the study of Supaprom, & Baimai (2004) and Donsakul (2009).

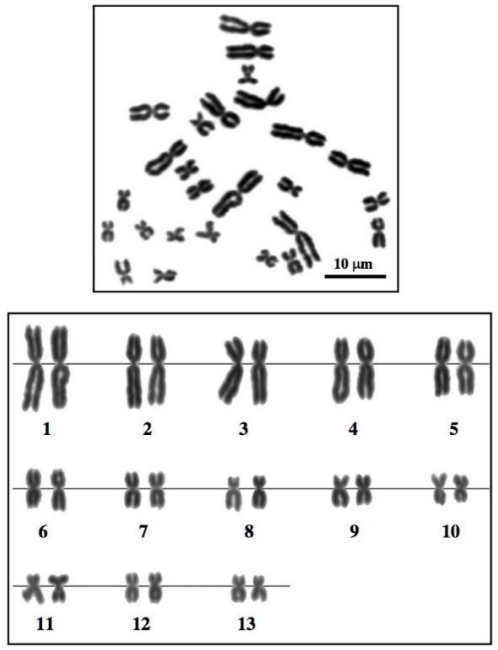

Figure 2 Metaphase chromosome plate and karyotype of male rough-skinned floating frog (*Occidozyga lima*), 2*n*=26 by conventional straining technique.

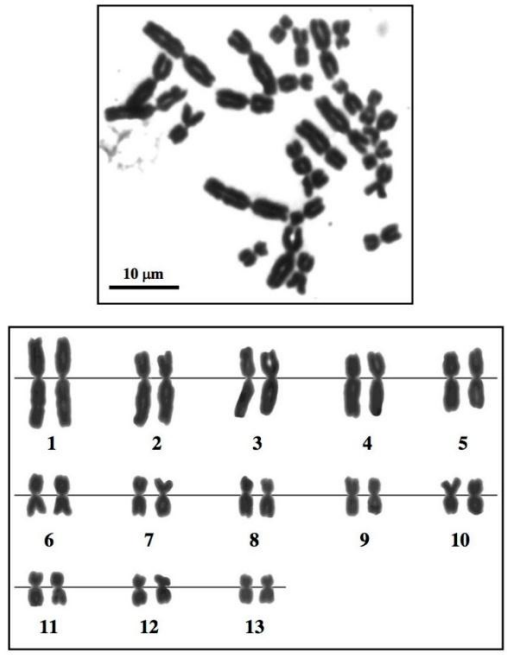

Figure 3 Metaphase chromosome plate and karyotype of female rough-skinned floating frog (*Occidozyga lima*), 2*n*=26 by conventional straining technique.

The types of chromosomes were 14 metacentric, and 12 submetacentric chromosomes. It is not consistent with the report of Supaprom, & Baimai (2004), which revealed that the chromosomes of *O. lima* are as following; 12 metacentric, and 14 submetacentric chromosomes. This is inconsistent with the report of Donsakul (2009), which revealed that *O. lima* has 18 metacentric and 8 submetacentric chromosomes. The cause of the differences between studies may be due to the use of different criteria for classification of chromosome types.

No cytologically distinguishable sex chromosomes were observed which is similar to the results from Supaprom, & Baimai (2004) and Donsakul (2009) and the *O. martensii* (Supaprom, & Baimai, 2004; Donsakul, & Rangsiruji, 2005). It is possible that the frog's sex-chromosomes are dependent on an initiation of differentiation. Therefore, chromosomes containing sex-determination gene cannot be found by cytogenetic analyses.

Our present study, was accomplished by using the Ag-NOR staining technique. The objective of this technique was to present nucleolar organizer regions (NORs) representing the location of genes that function in ribosome synthesis. NORs produce numerous gene expressions and contain more non-histone protein than other chromosome regions (Sharma, Tripathi, & Sharma, 2002; Khakhong et al., 2014). The region adjacent to the sub-centromeric regions of the long arm on chromosome pair 10 (small metacentric chromosomes) has observable NORs (Figure 4 and 5).

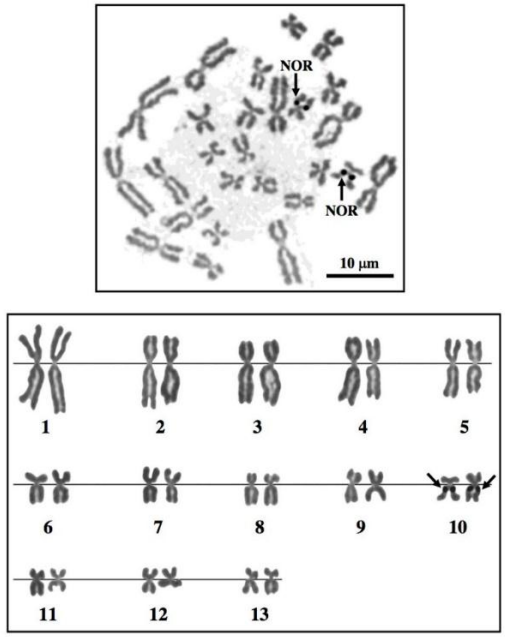

Figure 4 Metaphase chromosome plate and karyotype of male rough-skinned floating frog (*Occidozyga lima*), 2*n*=26 by Ag-NOR banding technique, arrows indicate NOR-bearing chromosomes.

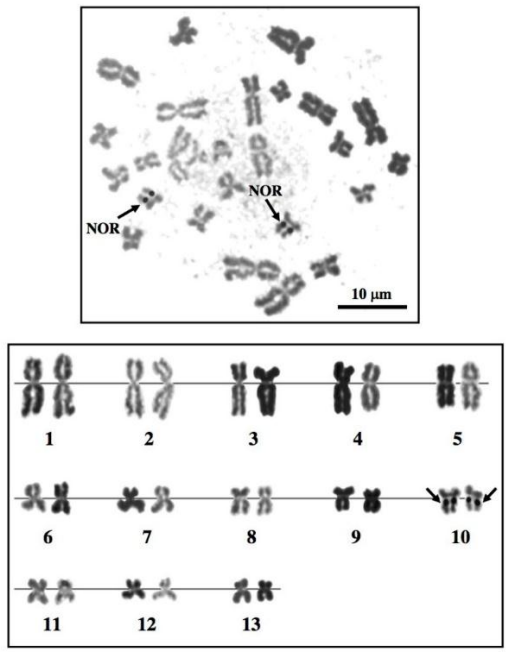

Figure 5 Metaphase chromosome plate and karyotype of female rough-skinned floating frog (*Occidozyga lima*), 2*n*=26 by Ag-NOR banding technique, arrows indicate NOR-bearing chromosomes.

Thus, the extra characteristic of long arms near the centromere of the chromosome pair 10 could be representative of the chromosome marker. This is similar to the published of Supaprom, & Baimai (2004) and Donsakul, & Rangsiruji (2005) that showed a pair of NORs on long arm in the *O. martensii*. Most species of amphibians have been examined with the Ag-NORs staining technique. In addition, the amount and location of NOR could provide an explanation for the evolution of each chromosome. Normally, most amphibians have only one pair of NORs on the chromosome. Furthermore, NORs are usually located close to the centromere of the chromosome arm.

The present study for meiotic cell division of *O. lima* found that during metaphase I (reductional division) (Figure 6J) the homologous chromosomes showed synapsis, which can be defined as the 13 ring bivalent (8 large bivalents and 5 small bivalents) and 13 haploid chromosomes at metaphase II (equational division) (Figure 6K) as the half of diploid species (2*n*=26).

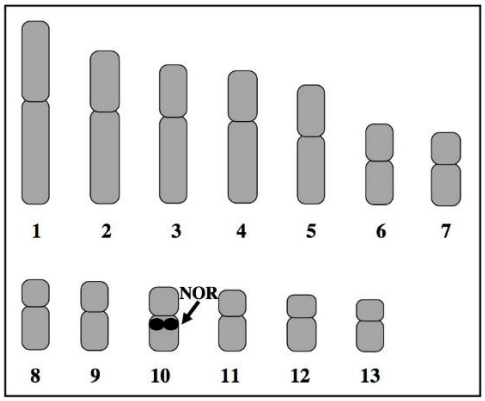

Figure 7 Idiogram showing lengths and shapes of chromosomes of the rough-skinned floating frog (*Occidozyga lima*), 2*n*=26 by Ag-NOR banding technique. The arrow indicates satellite chromosomes /NOR pair 10.

The largest metacentric chromosome pair 1 is the largest bivalent and smallest metacentric chromosome pair 13 is the smallest bivalent. No diakinesis and metaphase I cells with partially paired bivalents that are speculated to be heteromorphic sex-chromosomes. In interphase (Figure 6A and 6B) and prophase I (meiosis I), we found that *O. lima* had the distinctness of the observable leptotene (initiation of chromosome shrinking, Figure 6), zygotene (initiation of chromosome synapsis, Figure 6D), pachytene (completion of chromosome synapsis, Figure 6E), early diplotene (chiasma and crossing over,

Figure 6F and 6G) and late diakinesis (terminalisation, Figure 6H).

We have shown that the symmetrical karyotype of *O. lima*, which has two types of metacentric and submetacentric chromosomes is an important chromosome marker. Figure 7 shows the idiogram from Ag-NOR banding technique.

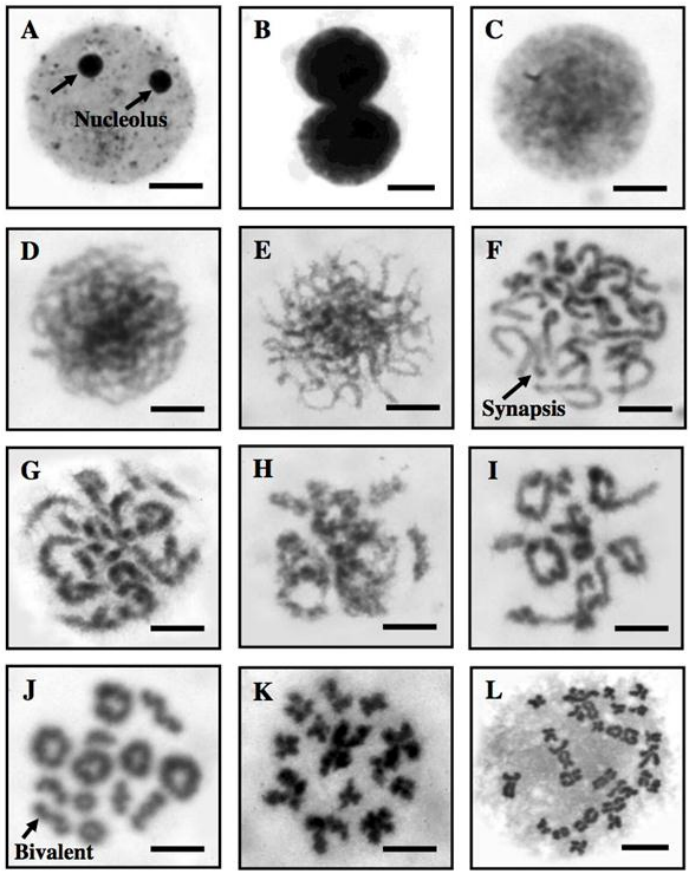

Figure 6 Meiotic cell division of the rough-skinned floating frog (*Occidozyga lima*), 2*n*=26 on interphase (A and B), leptotene (C), zygotene (D), pachytene (E), early diplotene (F and G), early diakinesis (H), late diakinesis (I), metaphase I (J), metaphase II (K), and metaphase of mitosis (L). Scale bars indicate 10 micrometres.

The idiogram shows gradually decreasing length of the chromosomes. The largest and smallest chromosomes show an approximately three folds size difference. Data of the chromosomal

checks on mitotic metaphase cells are shown in Table 2.

Table 2

Mean length of the short arm chromosome (Ls), long arm chromosome (Ll), and total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosomes in 20 cells of the male and female rough-skinned floating frog (*Occidozyga lima*), 2*n*=26.

Chro. pair	Ls	Ll	LT	RL±SD	CI±SD	Chro. size	Chro. type
1	2.322	3.018	5.341	0.145±0.004	0.566±0.013	Large	Metacentric
2	1.755	2.680	4.435	0.121±0.005	0.605±0.021	Large	Submetacentric
3	1.558	2.568	4.126	0.112±0.003	0.623±0.018	Large	Submetacentric
4	1.471	2.434	3.905	0.106±0.002	0.623±0.023	Large	Submetacentric
5	1.495	2.006	3.501	0.095±0.002	0.575±0.029	Large	Metacentric
6	1.066	1.310	2.376	0.065±0.002	0.550±0.017	Small	Metacentric
7	0.936	1.190	2.126	0.058±0.003	0.559±0.023	Small	Metacentric
8	0.765	1.278	2.043	0.056±0.003	0.625±0.022	Small	Submetacentric
9	0.855	1.114	1.970	0.054±0.005	0.567±0.015	Small	Metacentric
10*	0.832	1.014	1.846	0.050±0.003	0.549±0.018	Small	Metacentric
11	0.706	1.115	1.821	0.050±0.003	0.613±0.033	Small	Metacentric
12	0.685	1.017	1.701	0.046±0.003	0.609±0.030	Small	Submetacentric
13	0.636	0.928	1.564	0.042±0.003	0.592±0.040	Small	Metacentric

Remarks: * = NOR-bearing chromosome and chro. = chromosome.

Regarding, the chromosome marker of *O. lima*, chromosome pair one is the largest metacentric and chromosome pair 13 is the smallest metacentric chromosome. The karyotype formula of *O. lima* is as follows:

$$2n\ (26) = L^{m}_{4} + L^{sm}_{6} + S^{m}_{10} + S^{sm}_{6}$$

Acknowledgment

This work has supported by Department of Fundamental Science, Faculty of Science and Technology, Surindra Rajabhat University, Surin, Mueang 32000, Thailand

References

Donsakul, T. (2009). Karyotype from liver cells of seven amphibians (Amphibian, Anura) from Thailand. *Journal of Science and Technology Mahasarakham University*, 28: 162-170.

Donsakul, T., & Rangsiruji, A. (2005). Liver karyotypes in *Limnonectes blythii*, *Rana erythraea*, *Rana leptoglossa*, *Occidozyga martensii* and *Glyphoglossus molossus* (Amphibia, Anura). In: Proceedings of 43rd Kasetsart University Annual Conference: Veterinary Medicine and Science. Bangkok, p. 544-551.

Howell, W.M., & Black, D.A. (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia,* 36: 1014-1015.

Khakhong, S. et al. (2014). A first chromosomal characteristic of NORs in splendid snakehead fish, *Channa lucius* (Perciformes, Channidae). *Cytologia 79*(2): 133-139.

Patawang, I. et al. (2014). The identification of sex-chromosomes and karyological analysis of rice frog, *Fejervarya limnocharis*, (Anura, Ranidae) from northeast Thailand. *Cytologia 79*(2): 141-150.

Rooney, D. E. (2001). Human Cytogenetics: Constutitional Analysis: A Practical Approach. Oxford University Press, London.

Sangpakdee, W., Phimphan, S., Tengjaroenkul, B., Pinthong, K., Neeratanaphan, L., & Tanomtong, A. (2017). Cytogenetic study of three microhylid species (Anura, Microhylidae) from Thailand. *Cytologia* (Special Issue) 82(1): 67-74.

Sharma, O.P., Tripathi, N.K., & Sharma, K.K. (2002). *A review of chromosome banding in fishes.* In: Sobti, R. C., Obe, G., and Athwal, R. S. (eds.), Some Aspects of Chromosome Structure and Functions. Narosa Publishing House, New Delhi, pp. 109-122.

Sing, A. K., & Banerjee, R. (2004). Chromosome diversity of Indian mammals, amphibians and reptiles. *Zoological Survey of India 102* (Part 3-4): 127-138.

Supaprom, T. & Baimai, V. (2004). Karyotypes of the ten species of ranid frogs (Anura: Ranidae) from Thailand. *Amphibia-Retilia* 25: 104-111

Turpin, R., & Lejeune, J. (1965). *Les Chromosomes Humains.* Gauthier-Villars, Paris.

van Dijk et al. (2004). *Occidozyga lima*. 2006 IUCN Red List of Threatened Species.