CHARACTERIZATION OF CHROMOSOMAL AND REPETITIVE ELEMENTS IN THE GENOME OF *RANA NIGROVITTATA* (ANURA, RANIDAE): REVEALED BY CLASSICAL AND MOLECULAR TECHNIQUES

S. PHIMPHAN [1]*,
S. AIUMSUMANG [1], A. TANOMTONG [2]

[1] Biology program, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, 67000, Thailand
[2] Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand
E-mail: joodoof@gmail.com, topthesun1978@hotmail.com, tanomtong@hotmail.com

Karyotype study and microsatellites pattern in the genome of Rana nigrovittata were studied, with the aim to provide a standard karyotype, chromosome marker and the distribution of repetitive DNA elements, informative knowledge of cytogenetics and evolutionary events. Here, we analyzed the karyotype structure and the distribution of repetitive DNA sequence in this species using conventional banding and Fluorescence in situ hybridization techniques. The ten specimens (five males and five females) were collected from Phitsanulok province, Thailand. Mitotic metaphases were prepared from the bone marrows by the standard protocol. The result showed that R. nigrovittata had the diploid chromosome number of 2n = 26 and the fundamental number (NF) were 52 in both males and females. The karyotypes compose of six large metacentric, four large submetacentric, two medium metacentric, two medium submetacentric and 12 small submetacentric chromosomes. No sex related chromosome heteromorphism was observed in male (XY) or female (ZW) of this species. The NOR was observed in subcentromeric region on chromosome no 11. The C-positive heterochromatin blocks are mainly distributed in the centromere of most chromosomes, while some additionally in paracentromeric and telomeric regions. The large heterochromatic blocks were found on chromosome no 6. Some of repetitive elements were scattered while some were specific in the karyotype. The combine of conventional banding and molecular cytogenetics provide information for a cytogenetic determination of the examined species.

Key words: *Rana nigrovittata, karyotype, microsatellites, chromosome.*

ХАРАКТЕРИЗУВАННЯ ХРОМОСОМНИХ І ПОВТОРЮВАНИХ ЕЛЕМЕНТІВ У ГЕНОМІ RANA NIGROVITTATA (ANURA, RANIDAE) ЗА ДОПОМОГОЮ КЛАСИЧНИХ ТА МОЛЕКУЛЯРНИХ ТЕХНОЛОГІЙ

Дослідження каріотипу і структури мікросателітів у геномі *Rana nigrovittata* проводили з метою встановлення стандартного каріотипу, хромосомного маркера і розподілу повторюваних ДНК елементів, інформації щодо цитогенетики і еволюційних подій. У цій роботі ми проаналізували структуру каріотипу і поширення повторюваної послідовності ДНК у цьому виді, використовуючи традиційне пофарбування хромосом і флуоресцентну гібридизацію *in situ*. Десять особин (п'ять самців і п'ять самок) відібрали у провінції Пхітсанулок, Таїланд. Мітотичні метафази приготували з спинного мозку за стандартним протоколом. Результати продемонстрували, що *R. nigrovittata* мала диплоїдний набір хромосом ($2n = 26$), а фундаментальне число (NF) становило 52 як у самців, так і в самок. Каріотипи складалися з шести великих метацентричних, чотирьох великих субметацентричних, двох середніх метацентричних, двох середніх субметацентричних і 12 малих субметацентричних хромосом. У цього виду не спостерігали статево-обумовленого гетероморфізму хромосом у самців (XY) чи самок (ZW). NOR було виявлено у субцентромерній ділянці на хромосомі 11. В основному, С-позитивні блоки гетерохроматину розташовувалися в центромерній частині більшості хромосом, хоча деякі були додатково присутні у парацентромерних та теломерних ділянках. Великі блоки гетерохроматину було виявлено на хромосомі 6. Деякі повторювані елементи були розсіяні, хоча декілька були специфічними для каріотипу. Поєднання методів традиційного пофарбування хромосом і молекулярної цитогенетики дозволило отримати інформацію для цитогенетичного визначення досліджуваного виду.

Ключові слова: *Rana nigrovittata*, каріотип, мікросателіти, хромосома.

REFERENCES

Beccari N. (1926) Le Nombre des chromosomes dans les cellules genetales de *Bufo viridis*. CR Assoc Anat (21):29–31

Birstein VJ. (1984) Localization of NORs in karyotypes of four *Rana* species. Genetica 64:149–154. https://doi.org/10.1007/BF00115338

Blommers-Schlösser RMA. (1978) Cytotaxonomy of the Ranidae, *Rhacophoridae, Hyperoliidae* (Anura) from Madagascar with a note on the karyotype of two

amphibians of the Seychelles. Genetica 48:23–40. https://doi.org/10.1007/BF00125283

Chan-Ard T, Grossmann W, Gumprecht A et al. (1999) *Amphibians and Reptiles of Peninsular Malaysia and Thailand (an illustrated checklist)*, Bushmaster Publications. Wuerselen, Germany, 240 pp

Chaiyasut K. (1989) *Cytogenetics and cytotaxonomy of the family Zephyranthes*. Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok [in Thai], 1989

Cioffi MBP., Bertollo LAC. (2012) Chromosomal distribution and evolution of repetitive DNAs in Fish. In: Garrido, R. editor. Repetitive DNAs genome dynamics, Basel: Karger 7:197–221. https://doi.org/10.1159/000337950

Frost DR. (ed.) (1985) *Amphibian species of the world: A taxonomic and geographical reference*. Allen Press, Lawrence, Kansas

Guillemin C. (1967) Karyotypes de *Rana temporaria* (L.) et de *Rana dalmatina* (Bonaparte). Chromosoma 21:189–197. https://doi.org/10.1007/BF00343644

Heppich S. (1978) Hybridogenesis in *Rana esculenta*: C-band karyotypes of *Rana ridibunda*, *Rana lessonae* and *Rana esculenta*. Z Zool Syst Evolut Forsch 16:27–39. https://doi.org/10.1111/j.1439-0469.1978.tb00918.x

Heppich S, Tunner HG. (1979) Chromosomal constitution and C-banding in homotypic *Rana esculenta* crosses. Mitt Zool Mus Berlin 55:111–114. https://doi.org/10.1007/BF00273874

Hills DM, Green DM. (1990) Evolutionary changes of heterogametic sex in the phylogenetics history of amphibians. J Evol Biol 3:49–64. https://doi.org/10.1046/j.1420-9101.1990.3010049.x

Howell WM, Black DA. (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015. https://doi.org/10.1007/BF01953855

Iizuka K. (1989) Constitutive heterochromatin and nucleolus organizer regions in Japanese brown frogs, *Rana japonica* and *Rana ornativentris*. Jap J Herpetol 13:15–20

Joshy HSS, Kuramoto M, Sreepada KS et al. (2006) Karyotypic Variations in Three Indian Species of the Genus *Rana* (Anura: Ranidae) from the Western Ghats, India. Cytologia 71(1):63–68. https://doi.org/10.1508/cytologia.71.63

Khonsue W, Thirakhupt K. (2001) A checklist of the amphibians in Thailand. Nat Hist J Chulalongkorn Univ 1:69–82

Kuramoto M. (1972) Karyotypes of the six species of frogs native to the Ryukyu islands. Caryologia 25(4):547–559. https://doi.org/10.1080/00087114.1972.10796509

Kuramoto M. (1979) Karyotypes of several frogs from Korea, Taiwan and the Philippines. Experientia 39: 826–828. https://doi.org/10.1007/BF01978594

Kuramoto M. (1980) Karyotypes of several frogs from Korea, Taiwan and the Philippines. Experientia 36: 826–828. https://doi.org/10.1007/BF01978594

Kuramoto M. (1989) Karyological studies on some Philippine frogs. In: Matsui M, Hidika T, Goris RC eds. Current Herpetology in East Asia. Herpetol Soc Japan, Kyoto, 115–121 pp

Kuramoto M. (1990) A list of chromosome numbers of anuran amphibians. Bull Fukuoka Univ Educ 39: 83–127

Kuramoto M, Yong H-S. (1992) Karyotypes from several frog species from peninsular Malaya. *Herpetologica* 28(4):434–438

Liu W, Zan R. (1984) A special karyotype in the genus *Rana* – an investigation of the karyotype, C-banding and Ag-stained NORs of *Rana phrynodes* Boulenger. Acta Genetica Sinica 11:52–60. https://doi.org/10.3897/CompCytogen.v8i4.7623

Matsui M, Nishikawa K, Khonsue W et al. Allozymic variation in *Rana nigrovittata* (Amphibia: Anura) within Thailand with special reference to the taxonomic status of *R. mortenseni*. Nat Hist J Cu 2001 1(1):15–22

Matsui M, Hidetoshi O, Michael WL et al. (1995) Cytotaxonomic Studies of Three Ranid Species (Amphibia: Anura) from Hong Kong. JPN J Herpetol 16(1):12–18. https://doi.org/10.5358/hsj1972.16.1_12

Miura I. (1994) Sex chromosome differentiation in the Japanese brown frog, *Rana japonica*. Sex-related heteromorphism of the distribution pattern of constitutive heterochromatin in chromosome no. 4 of the Wakuya population. Zool Sci 11:797–806

Odierna G, Vences M, Aprea G et al. (2001) Chromosome data for Malagasy poison frogs (Amphibia: Ranidae: Mantella) and their bearing on the taxonomy and phylogeny. Zool Sci 18(4):505–514. https://doi.org/10.2108/zsj.18.505

Pinkel D, Straume T, Gray J. (1986) Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83(9):2934–2938. https://doi.org/10.1073/pnas.83.9.2934

Popov P, Dimitrov B. (1999) Karyotype study of *Rana camerani* and comparisons with the other 26-chromosome European brown frog species (Amphibia, Anura). Cytobios 97:13–22

Sangpakdee W, Phimphan S, Tengjaroenkul B et al. (2017) Cytogenetic Study of Three Microhylid Species (Anura, Microhylidae) from Thailand. Cytologia 82(1, Special Issue):67–74. https://doi.org/10.1508/cytologia.82.67

Seto T. (1965) Cytogenetic studies in lower vertebrates II.

Karyological studies of several species of frogs (Ranidae). Cytologia 30:437–446. https://doi.org/10.1508/cytologia.30.437

Schmid M. (1978) Chromosome banding in Amphibia II. Constitutive heterochromatin and nucleolus organizer regions in Ranidae, Microhylidae and Rhacophoridae. Chromosoma 68:131–148. https://doi.org/10.1007/BF00287145

Schmid M. (1980) Chromosome banding in amphibia IV. Differentiation GC- and AT-rich chromosome regions in Anura. Chromosoma 77(1):215–234. https://doi.org/10.1007/BF00292043

Schmid M, Steinlein C, Freiedl R et al. (1990) Chromosome banding in Amphibia. XV. Two types of Y chromosome heterochromatin hypervariability in *Gastrotheca pseustes* (Anura, Hylidae). Chromosoma 99:413–423

Shi HL, Zhang C, Wu M et al. (2006) A study on the karyotype, C-banding and Ag-NORs in *Rana nigromaculata*. Hereditas 28:533–539

Singh AK, Banerjee R. (2004) Chromosomal diversity of Indian mammals, amphibians and reptiles. Rec Zool Surv India 102(Part 3–4):127–138

Spasić-Bošković O, Tanić N, Blagojević J et al. (1997) Comparative cytogenetic analysis of European brown frogs: *Rana temporaria*, *R. dalmatina* and *R. graeca*. Cytologia 50(2):139–149. https://doi.org/10.1080/00087114.1997.10797393

Stohler R. (1927) Cytologische Untersuchungen an den Keimdrusen der mittel europaischen Kroten (*Bufo viridis* Laur., *B. calamita* Laur. and *B. vulgaris* Laur.). Z Zellforsch 7:400–475. https://doi.org/10.1007/BF00372490

Supaprom T. (2003) *Cytogenetics of Amphibians in Thailand*. Ph.D. Dissertation, Mahidol university [in Thai].

Supaprom T, Chantree P, Palasarn W. (1999) *Cytogenetics and cytotaxonomy of localized Amphibians in Northeastern Thailand*. Research project. Ubonratchathani university [in Thai].

Supaprom T, Baimai V. (2004) Karyotypes of ten species of Ranid frogs (Anura: Ranidae) from Thailand. Amphibia-Reptilia 25(1)104–111

Sumner AT. (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75(1):304–306. https://doi.org/10.1016/0014-4827(72)90558-7

Tautz D, Renz M. (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucl Acids Res 12(10)4127–4138. https://doi.org/10.1093/nar/12.10.4127

Received May 14, 2020
Received August 18, 2020
Accepted November 18, 2021