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Differences in the Number and Karyotypes of Chromosomes in the *Anura* Order in Indonesia

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Abstract

The diversity of organisms can be studied at the cellular level. Cytological data related to specific organisms can provide valuable information for their classification. One such method is examining the number and karyotypes of chromosomes in these organisms. A karyotype refers to the chromosomal phenotype, including a detailed structural description. Correspondingly, this study investigated differences in the number and karyotypes of chromosomes in species belonging to the order *Anura* in Indonesia. The method used was a literature review of various studies on the chromosomes of species within the order *Anura*. Based on the analysis, differences in the number and karyotypes of several species in the order *Anura* were identified. The species examined in this study included *Rana rufipes*, *Rana parvaccola*, *Huia sumatrana*, *Fejervarya limnocharis*, *Limnonectes cf. grunniens*, *Limnonectes cf. modestus*, *Fejervarya cancrivora*, *Limnonectes blythii*, *Polypedates celebensis*, *Polypedates leucomystax*, and *Microhyla pulchra*, which belong to the families *Ranidae*, *Dicroglossidae*, *Rhacophoridae*, and *Microhylidae*. The variation in the number of diploid chromosomes among the organisms studied ranged from 22 to 26 chromosomes. In addition to differences in chromosome numbers, variations in karyotypes were also observed across the studied organisms.

Keywords: *Anura*, chromosome number, chromosome karyotype

Introduction

According to the global list of countries with the largest biodiversity, Indonesia ranks second after Brazil. This is due to Indonesia's strategically advantageous geographical position, encompassing diverse habitats, unique compositions, rich flora and fauna, as well as endemic plants and animals (Nilawati et al., 2019). Anurans' diversity in Indonesia, consisting of ten families, spans the islands of Sumatra, Java, Kalimantan, Sulawesi, and Papua (Rafi et al., 2022).

The distribution of the order *Anura* is very broad; it can be found on land and in freshwater environments, residential areas, along rivers and waterways, on trees, and in both primary and secondary forests (Rohadian, 2022). Water bodies provide suitable habitats for anurans to breed (Carvalho-Rocha et al., 2020), as anurans are a group of animals that require water at various stages of their life cycle for growth and development (Prasetyo et al., 2020). Previous studies have shown that the quantity of vegetation positively correlates with the abundance of anuran

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species in tropical and temperate regions (da Silva et al., 2012).

Anurans possess morphological characteristics, such as very permeable skin, and exhibit complex, biphasic life cycles and geographically limited distribution patterns. The variation in the composition of anurans is influenced by factors such as available space, hydroperiod, vegetation cover, the type of surrounding matrix, and geomorphology. Thus, environmental factors significantly contribute to the taxonomic and functional diversity of the order *Anura* (Dalmolin et al., 2019).

Anurans play a key role in maintaining ecosystem stability by acting as predators of insects and their larvae. This helps reduce agricultural and plantation pests and controls insect populations that act as disease vectors (Ratna & Wijaya, 2013). Additionally, the order *Anura* serves as a biological indicator due to its sensitivity to changes in its habitat. As such, frogs and toads play an important role in maintaining ecosystem stability (Rohadian, 2022). In line with this, Erawan et al. (2021) demonstrated that amphibians are sensitive to environmental changes and can serve as indicators of biodiversity and local environmental threats, such as water pollution. The high sensitivity of amphibians also makes them useful bioindicators of contamination and environmental stress, one example being the biomagnification ability exhibited by frogs (Phoonaploy et al., 2016).

Environmental heterogeneity is one of the key factors contributing to species diversity (Silva et al., 2011). Species diversity is a crucial variable in conservation management (Rohadian, 2022). Several studies have shown that environmental complexity supports the formation of more microhabitats, thus increasing species diversity compared to homogeneous environments. Environmental heterogeneity is also a key parameter for the diversity of the order *Anura* (Silva et al., 2011). Furthermore, variations in topography and climate also play a role in increasing biodiversity across biomes. However, habitat fragmentation, habitat loss, climate change, and infectious diseases threaten the diversity of the order *Anura*, making it one of the most endangered vertebrate groups on Earth (Carvalho-Rocha et al., 2020). Like other animals, frogs and toads have specific environmental requirements for each species (Rohadian, 2022).

Several previous studies have documented the impact of habitat loss and changes on anuran biodiversity, though the results are inconsistent. Some studies indicate that land conversion makes areas no longer suitable for anuran reproduction and survival. For example, in Australia, the number of frog species has declined due to the conversion of Eucalyptus forests into pine monocultures. Other studies, however, show that even when areas are disturbed or converted, they can still support the lives of many indigenous anuran communities. Species resistant to environmental changes suggest that the negative impact of conversion on anuran abundance may decrease over time (Faruk et al., 2013). Research by Vasconcelos et al. (2018) suggests that climate change does not drastically alter the distribution of species richness gradients. However, it does impact the extinction or loss of species in biodiversity hotspots due to climatic incompatibility with many species.

A karyotype is a chromosomal phenotype that includes a structural description of chromosomes, such as their number, shape, centromere position, distribution of euchromatin and heterochromatin, and the size of the satellite. Chromosomes are arranged based on homologous pairs and sorted from longest to shortest according to centromere size and position (Tjong et al., 2012).

Based on the above explanation, the present study aimed to compile the results of previous studies on anuran diversity, focusing on the chromosomal observations of several species, to provide information about the chromosomal diversity within the *Anura* order.

Research Methods

This study employed a literature review method with data from previous studies examining various species belonging to the order *Anura*. The data was presented as a comparison table of chromosome counts, accompanied by images of chromosomal karyotypes for different species.

Research Results and Discussion

The species diversity of the order *Anura* identified in Indonesia comprised 450 species classified into 10 families. However, not all of these species were studied cytotaxonomically. Table 1 portrays the differences in the number of chromosomes in organisms of the order *Anura*.

Table 1
Differences in the Number of Chromosomes in Anura *Order Organisms*

No.	Species Name	Number of Diploid Chromosomes (2n)	Family
1	*Rana rufipes*	26	*Ranidae*
2	*Rana parvaccola*	26	*Ranidae*
3	*Huia sumatrana*	26	*Ranidae*
4	*Fejervarya limnocharis*	26	*Dicroglossidae*
5	*Limnonectes cf. grunniens*	24	*Dicroglossidae*
6	*Limnonectes cf. modestus*	24	*Dicroglossidae*
7	*Fejervarya cancrivora*	26	*Dicroglossidae*
8	*Limnonectes blythii*	24	*Dicroglossidae*
9	*Polypedates celebensis*	22	*Rhacophoridae*
10	*Polypedates leucomystax*	26	*Rhacophoridae*
11	*Microhyla pulchra*	24	*Microhylidae*

The *Ranidae* family is one of the most diverse groups of animals, distributed across most regions of the world with temperate and tropical climates. It can be found on almost all continents except Antarctica. This family exhibits remarkable diversity in ecology, morphology, and development across its vast geographical distribution (Huang et al., 2016). *Rana rufipes* and *Rana parvaccola* are species belonging to the *Ranidae* family. Based on research by Tjong et al. (2012), the diploid chromosome count for these two species is 26, with six pairs of large chromosomes and seven pairs of small chromosomes. Samples were taken from the Biological Education and Research Forest (*HPPB*) of Universitas Andalas, West Sumatra. The chromosome types of *R. rufipes* include metacentric chromosomes (12 pairs) and one pair of submetacentric chromosomes (chromosome 4). Figure 1 shows the metaphase and karyotype chromosomes of *R. rufipes*. On the other hand, the chromosome types of *R. parvaccola* include ten metacentric chromosomes and three submetacentric chromosomes located on chromosomes 4, 8, and 12. Figure 2 displays the metaphase and karyotype chromosomes of *R. parvaccola*.

Other species belonging to the *Ranidae* family found in Indonesia include *Huia sumatrana*. Research by Tjong et al. (2013) demonstrates differences in the karyotypes of *H. sumatrana* from different habitats. The diploid chromosome count of *H. sumatrana* is 26, consisting of six pairs of large chromosomes and seven pairs of small chromosomes. However, there are differences in the chromosomal karyotypes: *H. sumatrana* from the Padang region has submetacentric chromosomes on chromosomes 3 and 8, while *H. sumatrana* from the Pasaman region has a submetacentric chromosome only on chromosome 3. Figure 3 portrays the metaphase and karyotype chromosomes of *H. sumatrana* from Padang, while Figure 4 shows the metaphase and karyotype chromosomes of *H. sumatrana* from Pasaman.

The *Dicroglossidae* family is a group of amphibians with diversity and distribution centers in South Asia, Southeast Asia, East Asia, and islands in the shallow Sunda region and the Philippines. Members of this family are also found in northwestern and sub-Saharan Africa, as well as across the southern Arabian Peninsula to Pakistan (Köhler et al., 2021). In Indonesia, the *Dicroglossidae* family is found on the island of Java. It is divided into the subfamily *Dicroglossinae*, with 148 identified species, and *Occidozyginae*, with 22 species (Conlon, 2011). The genus *Limnonectes* is part of the *Dicroglossidae* family (Ginting et al., 2020). Likewise, the genus *Fejervarya*, originally belonging to the *Ranidae* family, was later reclassified as part of the *Dicroglossidae* family. Species of the genus *Fejervarya* include *Fejervarya cancrivora* and *Fejervarya limnocharis* (Akhsani et al., 2021).

Fejervarya limnocharis is a member of the *Dicroglossidae* family. Based on research by Djong et al., (2007), the diploid chromosome count for *Fejervarya limnocharis* is 26. The chromosomes consist of 13 bivalents, with five large-group chromosomes and eight small-group chromosomes. Figure 5 displays the metaphase and karyotype chromosomes of *Fejervarya limnocharis* from both males and females.

Limnonectes cf. grunniens, *Limnonectes cf. modestus*, and *Fejervarya cancrivora* are also members of the *Dicroglossidae* family. Research by Nasaruddin et al. (2009), with samples obtained from Kendari, Southeast Sulawesi, reveals that the diploid chromosome count for *Limnonectes cf. grunniens* and *Limnonectes cf. modestus* is 24. The chromosomes of *Limnonectes cf. grunniens* consist of five pairs of large chromosomes and seven pairs of small chromosomes. Twelve pairs of chromosomes are metacentric in type. Figure 6 shows the metaphase and karyotype chromosomes of *Limnonectes cf. grunniens*. *Limnonectes cf. modestus* consists of five pairs of large chromosomes with metacentric types and seven pairs of small chromosomes, with chromosomes 6-10 being metacentric and chromosomes 11-12 being telocentric. Figure 7 depicts the metaphase and karyotype chromosomes of *Limnonectes cf. modestus*. In contrast to these two species, the diploid chromosomes of *Fejervarya cancrivora* consist of 26 pairs of large chromosomes and karyotype chromosomes of *Limnonectes cf. modestus*. In contrast to these two species, the diploid chromosomes of *Fejervarya cancrivora* consist of 26 pairs with a distinct chromosomal composition.

Figure 1
Metaphase chromosomes and karyotype of R. rufipes*, 2n = 26. Source: Tjong et al. (2012).*

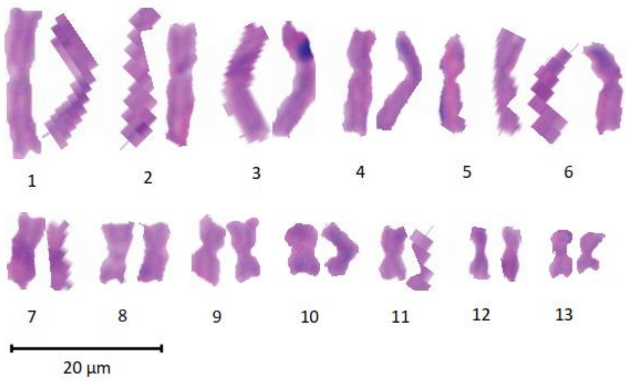


Figure 2
Metaphase chromosomes and karyotype of R. parvaccola*, 2n = 26. Source: Tjong et al. (2012).*

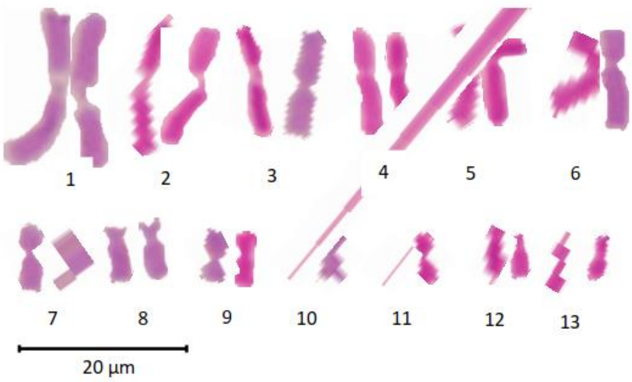


Figure 3
Metaphase chromosomes and karyotype of H. sumatrana *in Padang.*

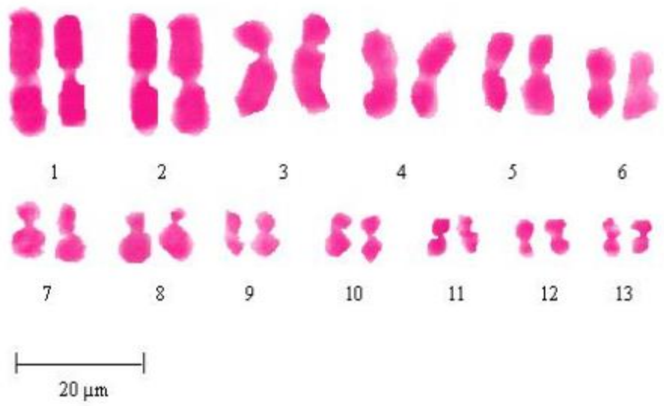


Figure 4
Metaphase chromosomes and karyotype of H. sumatrana *in Pasaman.*

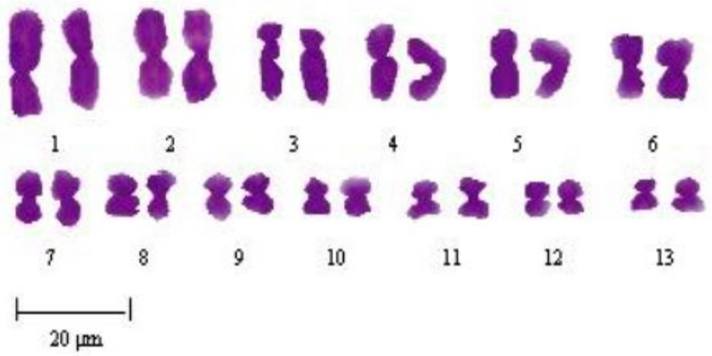


Figure 5
Metaphase chromosomes and karyotypes of Fejervarya limnocharis *males and females, 2n = 26, with conventional screening techniques. Source: Patawang et al. (2014).*

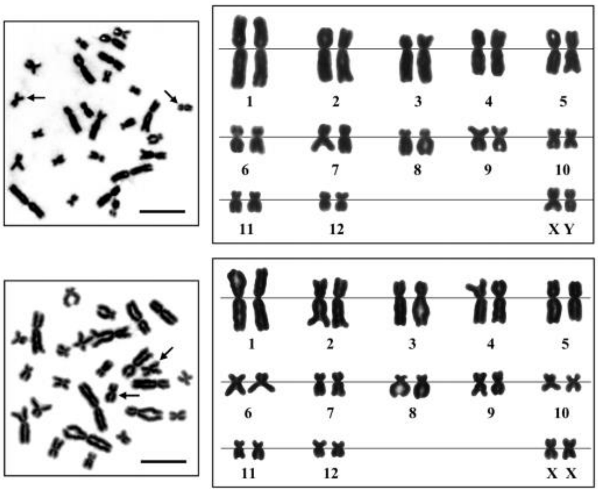


Figure 6
Metaphase chromosomes and karyotype of Limnonectes cf. grunniens*. Source: Nasaruddin et al. (2009).*

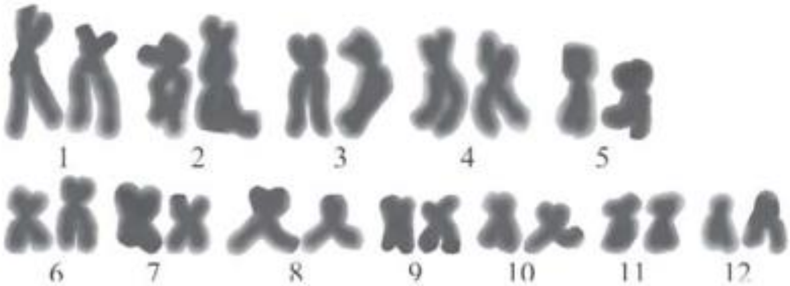


Figure 7
Metaphase chromosomes and karyotype of Limnonectes cf. modestus*. Source: Nasaruddin et al. (2009).*

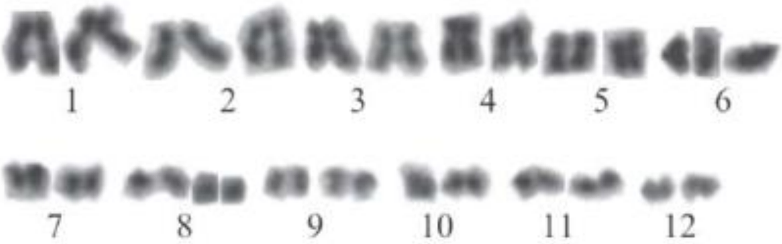


Figure 8
Metaphase chromosomes and karyotype of Fejervarya cancrivora*. Source: Nasaruddin et al. (2009).*

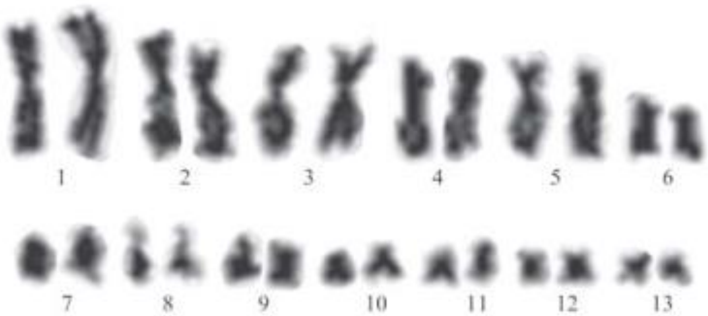


Figure 9
Metaphase chromosomes and karyotype of Limnonectes blythii*. Source: Phimphan et al. (2021).*

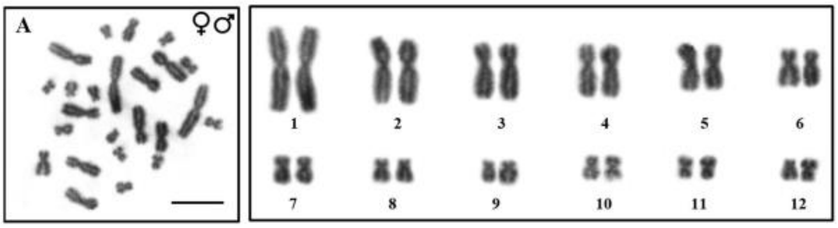


Figure 10
Metaphase chromosomes and karyotype of Polypedates celebensis*. Source: Nasaruddin et al. (2009).*

Figure 11
Metaphase chromosomes and karyotype of Polypedates leucomystax*. Source: Donsakul (2009).*

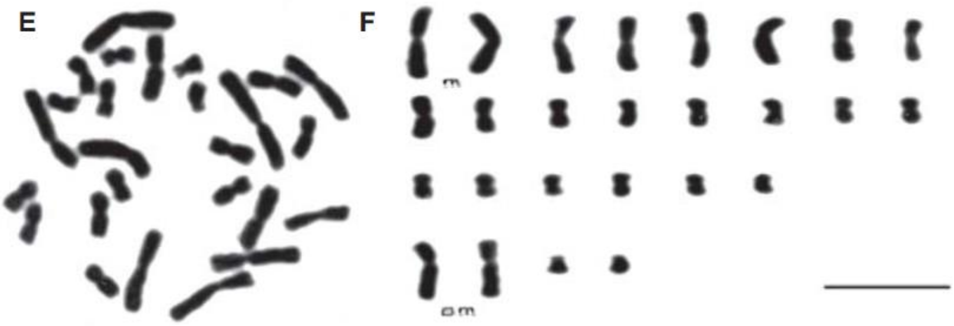
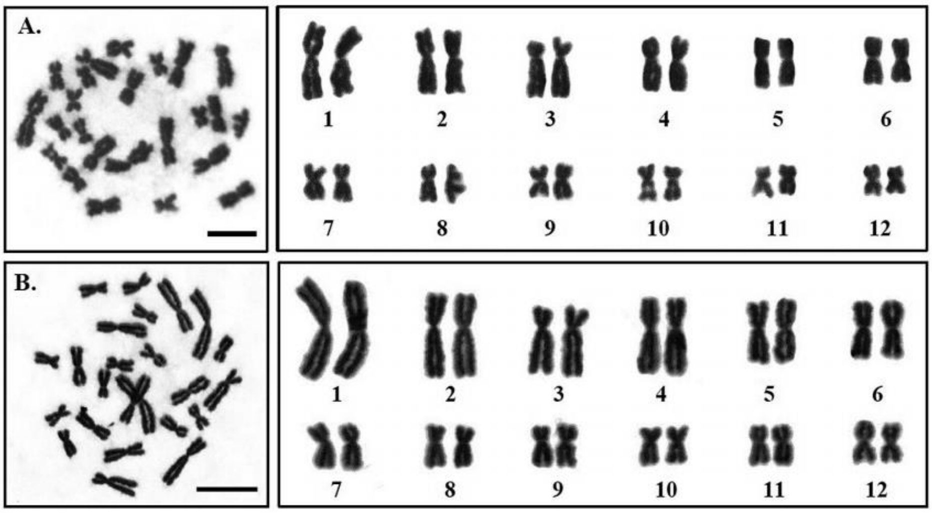


Figure 12
Metaphase chromosomes and karyotype of Microhyla pulchra*. Source: Sangpakdee et al. (2017).*



Limnonectes blythii is also a member of the family *Dicroglossidae*. Research by Ginting et al. (2020) identified the diversity of anurans in Deli Serdang Regency and found *L. blythii* around puddles with an ambient temperature of 23°C. *L. blythii* has 24 diploid chromosomes, consisting of four large metacentric chromosomes, four large submetacentric chromosomes, two medium metacentric chromosomes, and 14 small metacentric chromosomes (Phimphan et al., 2021). Figure 9 shows the metaphase chromosomes and karyotype of *L. blythii*.

The *Rhacophoridae* family is one of the *Anura* families with high diversity in Asia. The taxonomy of this family continues to evolve, and new species have been described (Onn et al., 2018). The diversity of *Rhacophoridae* on Earth accounts for 6% of the diversity of the order *Anura*. They are thought to have originated in the northeastern region of the mainland and the subtropical-subtropical islands of Asia, where they evolved approximately 53 to 68 million years ago (Ellepola & Meegaskumbura, 2023). The *Rhacophoridae* family, which consists of 416 species in 18 genera, is commonly known as the Old World Tree Frog (Jiang et al., 2019). One of the genera belonging to the *Rhacophoridae* family is the genus *Polypedates* (Hilmi et al., 2020).

Polypedates celebensis is one of the species in the genus *Polypedates*. Based on research by Nasaruddin et al. (2009), using samples taken from Kendari, Southeast Sulawesi, *P. celebensis* has 22 pairs of diploid chromosomes. These chromosomes are composed of five large pairs and six small pairs. They are divided into metacentric karyotypes (eight pairs) and submetacentric karyotypes (three pairs). Figure 10 displays the metaphase chromosomes and karyotype of *P. celebensis*.

Besides *P. celebensis*, *Polypedates leucomystax* also belongs to the *Rhacophoridae* family. Research by Yudha et al. (2019), carried out in the Paliyan Wildlife Sanctuary Area, Yogyakarta, successfully identified the presence of *P. leucomystax*. The study explained that several types of trees are suitable habitats for *P. leucomystax*. They have 26 diploid chromosomes, consisting of eleven pairs of metacentric chromosomes and two pairs of submetacentric chromosomes (Donsakul, 2009). Figure 11 displays the metaphase chromosomes and karyotype of *P. leucomystax*.

Microhylidae is the third-largest family in the order *Anura* (Targino et al., 2019). The species identified in this family include 400 species classified into 60 genera. *Microhylidae* species can be found worldwide, including in Asia, Africa, America, and Madagascar (Makowsky et al., 2009). The family consists of eleven subfamilies spread across various regions of the Earth's surface: *Asterophryinae*, *Cophylinae*, *Dyscophinae*, *Gastrophryninae*, *Hoplophryninae*, *Kalophryninae*, *Melanobatrachinae*, *Otophryninae*, *Phrynomerinae*, *Scaphiophryninae*, and *Microhylinae* (Matsui et al., 2011). One of the species in the subfamily *Microhylinae* is *Microhyla pulchra* (Targino et al., 2019).

M. pulchra is a species in the *Microhylidae* family found in Indonesia. Research by Yudha et al. (2023) identified the presence of *M. pulchra* in the Code River, Yogyakarta. The study explained that *M. pulchra* has a natural distribution in the upper reaches of the Code River. It has 24 diploid chromosomes, consisting of five large chromosomes, one medium chromosome, and six small chromosomes. Three large chromosomes are metacentric (chromosomes 1, 2, and 5), and two are submetacentric (chromosomes 3 and 4). The medium chromosome is metacentric (chromosome 6), and five small chromosomes are metacentric (chromosomes 7–11), while one small chromosome is submetacentric (chromosome 12). Figure 12 shows the metaphase chromosomes and karyotype of *M. pulchra*.

Differences in the number, size, and type of chromosomes of a species can be influenced by environmental factors such as geographical conditions, temperature, and regional altitude, leading to genetic variation (Tjong et al., 2013). Distinctions in chromosomal morphology can be observed at various levels, from different species to populations within a single species in the same environment. Environmental influences, both physical and chemical, can affect chromosomal changes during cell division and cause alterations in the gene composition of chromosomes (Saputra et al., 2014). Increasingly significant environmental changes can impact the cytogenetic characteristics of amphibians (Morescalchi, 1994). Thus, it can be understood that environmental changes may affect organisms at the genetic and chromosomal levels.

Conclusion

Indonesia's strategically important geographical position makes it a country with very high biodiversity. One example is the diversity within the order *Anura*. The diversity of *Anura* in Indonesia spans from the islands of Sumatra, Java, Kalimantan, and Sulawesi to Papua and includes ten different families. Organisms belonging to the order *Anura* exhibit differences in the number and karyotype of chromosomes. These chromosomal differences can be caused by various environmental factors, such as geographical conditions, temperature, and regional altitude, resulting in genetic variation.

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