



**MORPHOLOGICAL AND CHROMOSOMAL  
CHARACTERIZATION OF ORCHID *Peristylus goodyeroides*  
Lindl. FROM CURUG SETAWING, KULONPROGO**

**Febri Yuda Kurniawan<sup>1,3</sup>, Anindita Della Rosa Riyadi<sup>2,3</sup>**

<sup>1</sup> Study Program of Biotechnology, Graduate School, Universitas Gadjah Mada.

<sup>2</sup> Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada.

<sup>3</sup> Biology Orchid Study Club (BiOSC), Faculty of Biology,

Universitas Gadjah Mada, Indonesia

Email: febri.yuda.k@mail.ugm.ac.id

**DOI:** <http://dx.doi.org/10.26418/jpmipa.v12i2.41811>

**Abstract**

*Peristylus goodyeroides* is a terrestrial orchid that scattered around Southeast Asia. Morphological characters of *P. goodyeroides* can vary, depending on the ecological factors and habitat. Cytological characters in the form of chromosome configurations can be used as a taxonomic tool for the process of identifying and understanding variations in taxa. The purpose of this study was to determine the morphological characters and chromosome configuration of the *P. goodyeroides* from Curug Setawing, Kulonprogo. The method used was morphological characterization and plant chromosome preparation by squash method with the main steps of fixation, maceration, staining and observation. Data were analyzed with the help of Image raster 3, Corel Draw X7, and Microsoft Excel 2013. *P. goodyeroides* from Curug Setawing has the morphological characters of root tubers, cylindrical stems, ovate leaf shape, convolutive leaf arrangement and creamy white flowers. The orchid has a number of chromosomes  $2n = 10$  with a karyotype formula of  $2n = 2x = 8m + 2t$ . Metacentric chromosomes are found on chromosomes 1-8 and telocentric chromosomes 9-10. The absolute arm length of the chromosomes has a range of 2.03-3.44  $\mu\text{m}$ , the relative arm length of the chromosomes is 2.21-3.32  $\mu\text{m}$ , the length of the p arm is 1.13-1.58  $\mu\text{m}$  and the q arm is 1.23-2.12  $\mu\text{m}$ .

**Keywords:** chromosome, Curug Setawing, *Peristylus goodyeroides*, karyotype, morphology

**INTRODUCTION**

Orchids are flowering plants with high diversity and economic

value (Zhang et al., 2018; Pamarthi et al., 2019). Orchidaceae consists of 763 genera and 28,000 species



**Received** : 09/08/2020

**Revised** : 21/05/2021

**Accepted** : 05/06/2021

(Christenhusz & Byng, 2016). Curug Setawing is a tourist park area located in the Menoreh Mountains, Kulonprogo, Special Region of Yogyakarta. This area has latosol and grumusol types of soil (Nagara & Buchori, 2013), dry soil condition and humid air. There are 15 species of orchids found with three types of habitus, namely terrestrial, amoebophyte and epiphyte. One of the most attractive orchids found in this area is *Peristylus goodyeroides* with a total of 20 individuals and a frequency of 0.4 (Kurniawan et al., 2018). In addition to Kulonprogo, this species can also be found on the South Slope of Mount Merapi, Sleman Regency (Setiaji et al., 2018). *P. goodyeroides* is an orchid commonly found in teak forests and grasslands with an altitude of about 1,750 meters above sea level. The characters of this orchid are white flowers with a diameter of 7-8 mm. The leaves are 5-8 cm wide and up to 12 cm long (Comber, 1990; Nurfadilah, 2017). The phenotypic characters of *P. goodyeroides* appeared due to the interaction between genotype and environmental factors (Gienapp et al., 2017; Bocianowski et al., 2019).

Chromosomes are structures containing genetic material which have roles in the process of inheritance, growth and development of organisms (Shetty, 2018). The most common number of basic chromosomes (n) found in Orchidaceae are  $n = 19$ ,  $20$ , and  $21$  with  $2n = 38$ ,  $2n = 40$ , and  $2n = 42$  (Hartati et al., 2017).

The number of chromosomes in Orchidaceae varies, ranging from  $2n = 10$  to  $2n = 240$  (Sharma &

Mukai, 2015). Chromosome-based studies which have been carried out on the genus *Peristylus* include *P. constrictus* which has a number of chromosomes  $2n = 38$  and *P. coeloceras*  $2n = 42$  (Luo, 2004; Hartati et al., 2017).

Each species has significantly different shape and number of chromosomes, so has its karyotype. (Bolcun-Filas & Schimenti, 2012). Karyotype plays important role in providing information about taxonomic relationships, genetic abnormalities and the origin of a species (Young et al., 2012). Karyotype abnormalities can be related to abnormalities in the anatomical, morphological and physiological characteristics of an individual (Samatadze et al., 2020). Chromosome identification is carried out during metaphase or prometaphase based on cytometric images and morphometric data. This way is classified as conventional method (Ata et al., 2017).

Chromosome analysis has several basic functions. One of them are as an indication of the evolutionary process of a species based on the structure and number of chromosomes (Lusinska et al., 2019), species identification tools (Lukhtanov & Shapoval, 2017) and could determine the genetic diversity of species indicated by variations in the population (Bzdega et al., 2016).

*P. goodyeroides* is a natural orchid with botanical value. It is because the prospects of this species has not been deeply explored. In addition to Yogyakarta, this species can also be found in Purwodadi (East Java) (Nurfadilah, 2017), Maluku and Sulawesi (Nurfadilah, 2020).

This orchid can be found in tropical and subtropical regions in Asia, such as in India, Nepal, and so on (Jalal & Jayanthi 2015). Differences in geographic location and environmental factors play an important role in the phenotypic diversity of orchids (Djordjević et al., 2016; Erzurumlu et al., 2018).

*P. goodyeroides* found in Kulonprogo may have different morphological characters with other regions. Therefore it is necessary to carry out the taxonomic characterization of *P. goodyeroides* from Kulonprogo based on morphological and cytogenetic characters. Based on the literature

searching, there is no research has been found on cytogenetic data or the number of chromosomes in the orchid *P. goodyeroides* from Kulonprogo, so that this cytogenetic research could complete and enrich the taxonomic evidence on this orchid using cytotaxonomic data.

## **METHODS**

### **Time and Place of Research**

Sampling of *P. goodyeroides* was conducted in Curug Setawing, Kulonprogo, 654 m above sea level (7°44'24.3" SL; 110°08'44.3" EL) (Figure 1). This research was conducted in August-October 2019.

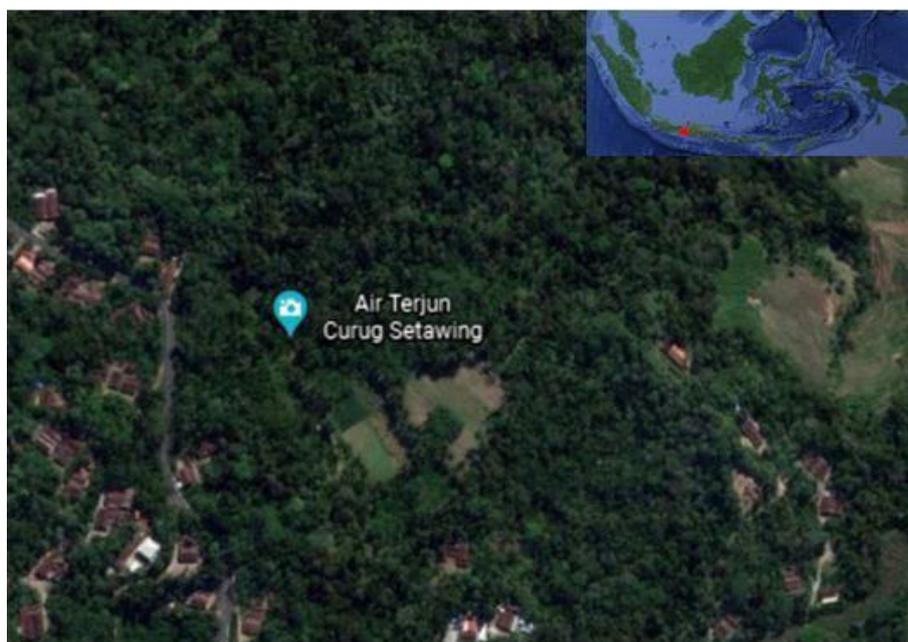


Figure 1. Orchid sampling location of *Peristylus goodyeroides* in Curug Setawing area, Kulonprogo (source: <https://earth.google.com>)

## **Procedures**

### **1. Morphological Characterization**

Morphological characterization was carried out by recording the morphological characters of orchids including habitus, root type, stem

shape, leaf shape, leaf tip shape, leaf size, leaf color and arrangement, type of flower, flower color, sepal and petal shapes and special characters on labellum. After the morphological characters obtained, identification of orchids is carried out by matching the

**Febri Yuda Kurniawan, Anindita Della Rosa Riyadi**

Morphological And Chromosomal Characterization Of Orchid *Peristylus Goodyeroides* Lindl. From Curug Setawing, Kulonprogo

morphological characters of the species with the Orchid of Java literature (Comber, 1990).

## 2. Chromosome Preparation

The preparation method used is the squash method (Aristya et al., 2015; Aristya et al., 2019) with modifications. The roots of orchid are washed with water before preparation. The root tips of the young *P. goodyeroides* orchids were cut  $\pm 5$  mm from the tip at 09.00 am. The root pieces were then immersed in a flakon bottle filled with distilled water for 24 hours, then placed in a refrigerator at 5° C. The root pieces were then washed with distilled water three times.

Fixation was conducted by adding 45% glacial acetic acid solution to the root sample. Then the samples were incubated at 4° C for  $\pm 30$  minutes in the refrigerator. The root pieces were then washed with distilled water three times. After that, maceration is carried out. The root sample was put into a flakon bottle then added with 1 N HCl and incubated in a block heater with a temperature of 62° C for  $\pm 30$  minutes. Staining was carried out with the help of a fulgen solution which was added to the root sample and then the sample was incubated at room temperature for 1 hour.

After the coloring stage, then the root pieces are squashed (squashing). The cut of purple root tip is placed on a glass preparation and glycerin is added, then covered with a cover glass. The root tip is then squashed (squashing) with the base of pen slowly until distributed. The preparations were then observed using a light microscope with a

magnification of 10x40. The variables observed included: number, size, chromosome shape and karyotype pattern (Hartati et al. 2014).

## 3. Visualization of chromosome shape and karyotyping

Visualization of chromosome shapes is assisted by Image raster 3 and Corel Draw X7 softwares. First, the chromosome shape is reconstructed with the help of the "shape tool" and "freehand tool" menus in Corel Draw X7. Second, labeling chromosomes with a temporary number is carried out using the "text tool" menu. Next, the chromosome arm length measurements (p and q arms) were carried out with the help of Image raster 3. The measurement results obtained were then used to calculate the absolute arm length, centromere index, chromosome arm length ratio, identification of chromosome shape, actual chromosome numbering, and manufacturing idiogram using Microsoft Excel 2013.

## 4. Data analysis

The determination of chromosomes number is known by calculating the number of chromosomes visualized at prometaphase. Karyotypes and idiograms are made using Corel Draw X7 software and Microsoft Excel 2013. According to Levan et al. (1964) the value of the Centromere Index (CI) can be calculated by the following formula:

$$CI = \frac{\text{Length of the short arm (p)}}{\text{Total length of chromosome (p+q)}} \times 100$$

The ratio value of long arm to short arm of chromosomes (CAR) can be calculated by the following

formula:

$$CAR = \frac{\text{Length of the long arm (q)}}{\text{Length of the short arm (p)}}$$

Based on the CI and CAR values obtained, then the chromosome shape can be determined which is classified into:

Table 1. The shape of chromosome based on centromere index and chromosome arms ratio

Chromosome shape	CI	CAR
Metacentric	37.5-50	1-1.68
Submetacentric	25-37.5	1.68-3
Subtelocentric	12.5-25	3.01-7
Telocentric	0-12.5	$\geq 7$

(Levan et al., 1964).

## RESULTS AND DISCUSSION

*Peristylus goodyeroides* collected from Curug Setawing, Kulonprogo is included in the subfamily Orchidoideae, tribe Orchideae, subtribe Orchidinae and genus *Peristylus* (Jin et al., 2017). *P.*

*goodyeroides* is  $\pm$  40-50 cm high, a perennial herbaceous plant and terrestrial type (Figure 2A). *P. goodyeroides* has adventitious roots with rhizomes. It has sympodial stem growth type with a cylindrical stem shape.



Figure 2. Morphological characters of *Peristylus goodyeroides*. Habitus of *Peristylus goodyeroides* (A), compound inflorescence (B) and single flowers (C). A and B bar = 5 cm, C bar = 0.1 cm.

It has yellowish green leaves, ovate with  $\pm$  18 cm long and  $\pm$  7 cm wide, convolutive arrangement of young leaves, flat leaf edges with pointed leaf tips. The leaves are terminal totaling 3-7 leaves. Alternate

leaves arrangement. Parallel leaf venation. Clustering (raceme) inflorescence (Figure 2B) with incomplete aperture.

It has creamy white flowers consisting of sepals, petals and

**Febri Yuda Kurniawan, Anindita Della Rosa Riyadi**

Morphological And Chromosomal Characterization Of Orchid *Peristylus Goodyeroides* Lindl. From Curug Setawing, Kulonprogo

labellum (Figure 2C) with respective characters are ovate-shaped dorsal sepalum, triangular lateral sepala with flat edges and pointed tips, lateral petala has a narrower base with a rounded tip and the edges are flat while on the lateral part it is shaped like a wing. There are parallel veins on the petals and sepals of the *P. goodyeroides* orchid. The labellum has three lobes that are tapered at the ends and narrower at the sides.

Based on morphological characters identified from samples collected, it is known that the species is *P. goodyeroides* according to the similarity of morphological characters in Comber (1990). Other species from the genus *Peristylus* such as *P. gracilis* have different morphological characteristics from *P. goodyeroides*. The difference lies in the loose leaf arrangement at the top of the stem and the smaller flower size with longer spurs than the sepals. *P. constrictus* has more oval sepal morphological character with a conduplicate-shaped dorsal sepalum and lateral sepala having irregular edges, the petala spreads upward but does not form a cap with the dorsal sepalum, the spura has a groove in the middle and has a short column structure. *P. coeloceras* has stem with a tubular sheath and opposite leaf arrangement, the leaves are elliptical and the petals are rhombic and oblique (Raskoti et al. 2017).

Based on morphological observation *P. goodyeroides* collected from Kulonprogo have the same characteristics as the literature. Although morphologically similar but could not be ascertained accurately that the sample is *P. goodyeroides*. Ecological factors can influence the

genotype of an individual as a form of adaptation to the environment. In natural populations, we often encounter the phenomenon of phenotypic plasticity as in behavior or morphology. This phenomenon causes the process in analyzing the interaction between genotype and phenotype becomes difficult (Gienapp et al., 2017). Besides the cryptic species also play a role in genetic variation accounted for although phenotypically alike. Therefore, it is necessary to have complementary data to support the hypothesis such as cytogenetic data.

Based on observation of *P. goodyeroides* chromosomes, it can be seen that the number of diploid chromosomes observed is 10 chromosomes ( $2n = 10$ ) (Figure 3). In Orchidaceae, there are variations in the number of chromosomes in each member of species. The number of diploid chromosomes  $2n = 10$  can be found in members of the subtribe Oncidiinae (Daviña et al., 2009). Members of the subfamily Spiranthoideae and Orchidoideae are known to have a varying number of chromosomes, ranging from  $n = 10$  to  $n = 50$ . In earlier studies, genus *Peristylus* has varied number of basic chromosomes between  $2n = 21, 23, 44,$  and  $18$  (Felix & Guerra, 2005). In other studies, it is stated that the variation in the number of chromosomes in this genus is  $2n = 28, 36, 38, 42, 44, 46$  and  $88$  (Luo, 2004).

Based on data obtained from The Chromosome Counts Database (CCDB) (Rice et al., 2015) it is known that there are variations in the number of chromosomes in *P. goodyeroides*, namely  $n = 47, 46, 42, 28, 23, 21$  (CCBD, 2021). That results obtained

from the researches conducted in the years 1968-1990. Conventional cytogenetic research uses cytometric images and morphometric data to reconstruct a karyogram (Ata et al.,

2017). According to Mártonfiová (2013), it is necessary for standardization in the measurement of the chromosome arm, because the value resulted was unstable.

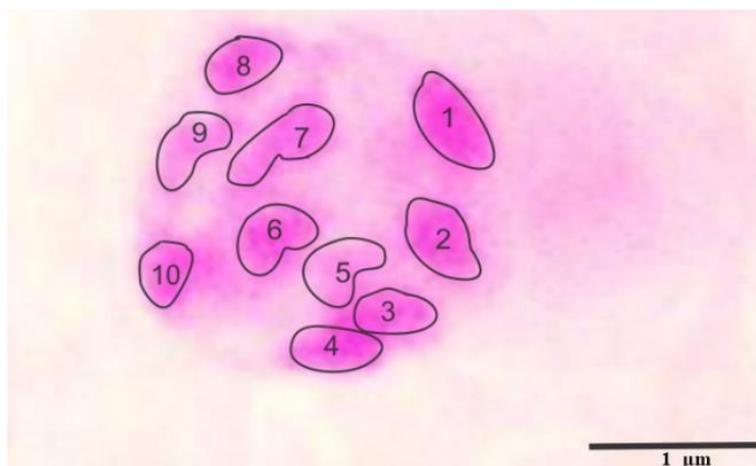


Figure 3. Visualization of chromosome *P. goodyeroides* observed at prometaphase. bar = 1  $\mu\text{m}$ .

The number of chromosomes can provide information about the genetic diversity of orchids in Indonesia. Such information can be used to determine the phylogenetic relationship between species of orchids in Indonesia (Bzdega et al., 2016). Based on literature studies, the number of chromosomes in *P. goodyeroides* species in Kulonprogo have not been reported before. Comparing with *P. constrictus*, the number of chromosomes in *P. constrictus*  $2n = 38$  (Hartati et al., 2017).

It can be used as a comparison to the number of chromosomes *P. goodyeroides* because both species are still classified in the same genus although each species can basically have a different number of chromosomes (Suliartini et al., 2004). In this study, the chromosome number of *P. goodyeroides* orchids was  $2n = 10$ . The difference in the number of

chromosomes between species can be caused by differences in geographical conditions, habitat, the domestication process, and so on. This difference can lead to a process of speciation and evolution. Products from a speciation will rise to new species of the same genus, but with a different chromosome configuration (Djordjevic et al., 2016; Erzurumlu et al., 2018). Not all variations in the number of chromosomes in a species necessarily lead to the speciation process. Many orchid species vary in the number of chromosomes, even within the same species. This variation may also arise due to the sample preparation is not exactly at the active division, instability measurement and so on. In essence, each species has a fixed number and structure of chromosomes. This consistency can be utilized for taxonomic purposes (Wang & Zheng, 2017). The differences in

chromosomes can describe the genetic variation in a species. The differences in morphological characters can describe biochemical variations which

are the products of gene expression and adaptation processes in their habitat (Hastuti et al., 2009).

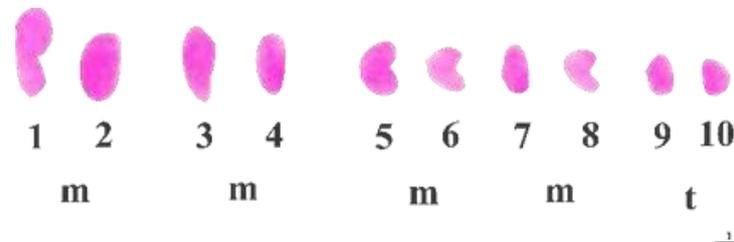


Figure 4. Chromosome karyotype of *P. goodyeroides* orchid from Kulonprogo. bar = 1 µm.

The process of arranging a karyotype and an idiogram is based on the appearance of chromosomes in the mitotic prometaphase phase. The arrangement of the karyotype is based on the shape, size and location of the centromere (Singh, 2003). Based on the results of the karyotype of *P. goodyeroides* (Figure 4), the karyotype formula for this orchid species is  $2n = 2x = 10 = 8m + 2t$  with

an absolute chromosome length range of around (2.03-3.44) µm. Based on the results of the karyotype visualized in the form of an idiogram, it can be seen that the chromosome pairs number 1-4 (chromosome number 1-8) are metacentric type and chromosome pair number 5 (chromosomes number 9 and 10) are telocentric type (Figures 4 and 5).

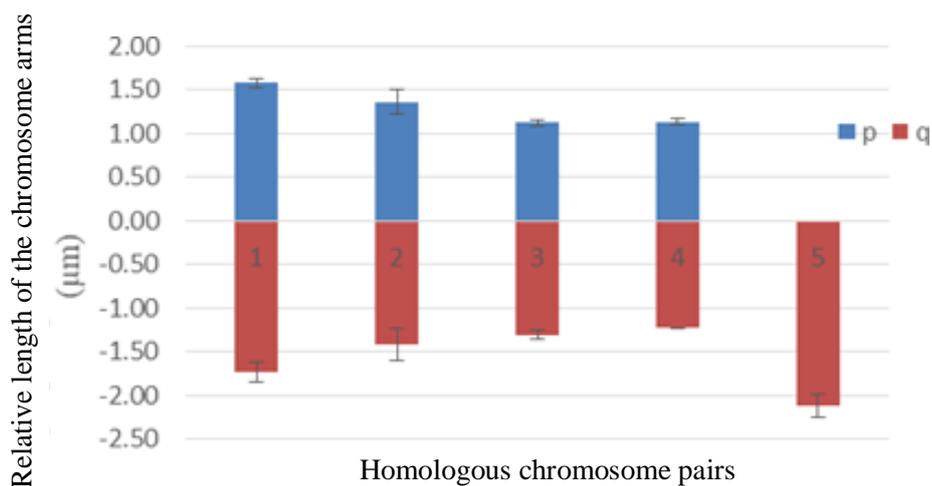


Figure 5. Chromosome idiogram of *P. goodyeroides* orchid

Based on the results of the karyotype analysis, *P. goodyeroides* orchid is classified as an asymmetric

karyotype because it has a variety of formulas consisting of metacentric and telocentric chromosomes. Asymmetric

karyotype is karyotype composed of chromosomes which have variations in shape and are considered the result of new cultivation (Singh, 2003). *P. goodyeroides* orchids have relative chromosome length ranging from 2.21-3.32  $\mu\text{m}$  with short arm length (p) ranging from 1.13-1.58  $\mu\text{m}$  and long arm chromosome (q) between 1.23-2.12  $\mu\text{m}$  (Figure 5).

### CONCLUSION

Based on the result of study, it can be concluded that the morphological characters observed in *P. goodyeroides* orchids are perennial herbaceous habitus, terrestrial orchids, root tubers, sympodial cylindrical stems, ovate leaf shape, convolutive leaf arrangement and creamy white flowers, ovate and triangular sepals with flat edges and pointed tips, wing-shaped petals with flat edges and rounded tips and also three lobes of labellum with pointed tips used as a characteristic for the orchid. The number of *P. goodyeroides* chromosomes observed is  $2n = 10$  with the karyotype formula  $2n = 2x = 10 = 8m + 2t$  which consists of five pairs of chromosomes with a metacentric (chromosome number 1-8) and telocentric (chromosome number 9-10). Molecular data is needed to strengthen the results obtained such as RAPD, ISSR, SSR, RFLP, sequencing, FISH (Flourescence In-Situ Hybridization), and other methods.

### REFERENCES

- Aristya, G.R., Daryono, B.S., Handayani, N. S.N., & Arisuryanti, T. (2015). *Karakterisasi kromosom tumbuhan dan hewan*. Gadjah Mada University Press. Yogyakarta.
- Aristya, G.R., Zuyyina, C., Febiansi, D., Ayuningsih, R., Prasiwi, K.D., Nurwijayanti, T.A., Mujahidah, U. & Renaldy, B. (2019). Karakterisasi kromosom spesies anggota Familia Solanaceae. *BIOTROPIC: The Journal of Tropical Biology* 3(1): 24-38.
- Ata, A.M., Abo-Bakr, M.A., El-Mazny, M.Y., Galal, A.A., Abd El-Twab, M.H., & Ramadan, A.M.M. (2017). A study of karyotype and mitotic chromosome behavior of some pea (*Pisum sativum* L.) genotypes cultivated in Egypt. *Minia Journal of Agriculture Research and Development* 37(4): 587-603.
- Bocianowski, J., Warzecha, T., Nowosad, K., & Bathelt, R. (2019). Genotype by environment interaction using AMMI model and estimation of additive and epistasis gene effects for 1000-kernel weight in spring barley (*Hordeum vulgare* L.). *Journal of Applied Genetics* 60(1): 127-135.
- Bolcun-Filas, E. & Schimenti, J.C. (2012). Genetics of Meiosis and Recombination in Mice. *International Review of Cell and Molecular Biology* 298(1): 179-227.
- Bzdega, K., Janiak, A., Książczyk, T., Lewandowska, A., Gancarek, M., Sliwinska, E., & Tokarska-

- Guzik, B. (2016). A survey of genetic variation and genome evolution within the invasive *Fallopia* complex. *PLOS ONE* 11(8): 1-23.
- CCBD. (2021). *The Chromosome Counts Database* (CCDB) version 1.58. Retrieved April 03, 2021, from <http://ccdb.tau.ac.il/>
- Christenhusz, M.J.M. & Byng, J.W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa* 261(3): 201–217.
- Comber, J.B. 1990. *Orchid of Java*. The Royal Botanic Garden Kew. London.
- Daviña, J.R., Grabiele, M., Cerutti, J.C., Hojsgaard, D.H., Almada, R.D., Insaurralde, I.S., & Honfi, A.I. (2009). Chromosome studies in Orchidaceae from Argentina. *Journal of Genetics and Molecular Biology* 32(4): 811–821.
- Djordjević, V., Tsiftsis, S., Lakušić, D., Jovanović, S., & Stevanović, V. (2016). Factors affecting the distribution and abundance of orchids in grasslands and herbaceous wetlands. *Systematics and Biodiversity* 14(4): 355-370.
- Erzurumlu, G.S., Sultana, N., Vural, M., & Serçe, S. (2018). Genetic and phenotypic variation among Turkish terrestrial orchid species as revealed by RAPD and morphological characteristics. *Turkish Journal of Agriculture and Forestry* 42(4): 227-236.
- Felix, L., & Guerra, M. (2005). Basic chromosome numbers of terrestrial orchids. *Plant Systematics and Evolution* 254(1): 131–148.
- Gienapp, P., Laine, V.N., Mateman, A.C., van Oers, K., & Visser, M.E. (2017). Environment-dependent genotype-phenotype associations in avian breeding time. *Frontiers in Genetics* 8(102): 1-9.
- Google Earth. (2020). *Maps of Curug Setawing location, Kulonprogo, Indonesia*. Retrieved December 23, 2020, from <https://earth.google.com/web/>.
- Hartati, S., Darsana, L., & Cahyono, O. (2014). Studi karakterisasi anggrek secara sitologi dalam rangka pelestarian plasma nutfah. *Jurnal Ilmu-Ilmu Pertanian XXIX* (1): 25-30.
- Hartati, S., Nandariyah, Yunus, A., & Djoar, D.W. (2017). Short communication: cytological studies on black orchid hybrid (*Coelogyne pandurata* Lindley). *Biodiversitas* 18(2): 555-559.
- Hastuti, D., Suranto, Setyono, P. (2009). Variation of morphology, karyotype and protein band pattern of adenium (*Adenium obesum*) varieties. *Bioscience* 1(2): 78-83.
- Jalal, J.S. & Jayanthi, J. (2015). An

- annotated checklist of the orchids of western Himalaya, India. *Lankesteriana* 15(1): 7-50.
- Jin, W.T., Schuiteman, A., Chase, M.W., Li, J.W., Chung, S.W., Hsu, T.C., & Jin, X.H. (2017). Phylogenetics of subtribe Orchidinae s.l. (Orchidaceae; Orchidoideae) based on seven markers (plastid *matK*, *psaB*, *rbcL*, *trnL-F*, *trnH-psba*, and nuclear nrITS, *Xdh*): implications for generic delimitation. *BMC Plant Biology* 17(222): 1-14.
- Kurniawan, F.Y., Setiaji, A., Putri, F., Suyoko, A., & Semiarti, E. (2018). Diversity and conservation strategy of orchids under anthropogenic influence in Taman Wisata Alam Curug Setawing, Yogyakarta. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia* 4(2):173-177.
- Levan, A., Fredga, K. & Sandberg, A.A. (1964). *Nomenclature for centromic position on chromosomes*. Institute of Genetics, Lund. Sweeden.
- Lukhtanov, V.A., & Shapoval, N.A. (2017). Chromosomal identification of cryptic species sharing their DNA barcodes: *Polyommatus* (*Agrodiaetus*) *antidolus* and *P. (A.) morgani* in Iran (*Lepidoptera*, *Lycaenidae*). *Comparative cytogenetics* 11(4): 759–768.
- Luo, Y.B. (2004). Cytological studies on some representative species of the Tribe Orchideae (Orchidaceae) from China. *Botanical Journal of the Linnean Society* 145(2): 231-238.
- Lusinska, J., Betekhtin, A., Lopez-Alvarez, D., Catalan, P., Jenkins, G., Wolny, E., & Hasterok, R. (2019). Comparatively barcoded chromosomes of *Brachypodium* perennials tell the story of their karyotype structure and evolution. *International journal of molecular sciences* 20(22): 1-19.
- Mártonfiová, L. (2013). A method of standardization of chromosome length measurement. *Caryologia* 66(4): 304-312.
- Nagara, R.P. & Buchori, I. (2013). Pembangunan model keruangan untuk identifikasi kawasan rawan erosi di wilayah pesisir (studi kasus: Kabupaten Kulonprogo). *Jurnal Teknik PWK*, 2(4): 948-957.
- Nurfadilah, S. (2017). Population study of *Peristylus goodyeroides* (Orchidaceae) in five habitats and implication for its conservation. *Biodiversitas* 18(3): 1084-1091.
- Nurfadilah, S. (2017). Utilization of orchids of Wallacea region and implication for conservation. *IOP Conference Series: Earth*

- and Environmental Science* 473(012063): 1-13.
- Pamarthi, R.K., Devadas, R., Rajkumar, Rai, D., Kiran-Babu, P., Meitei, A.L., De, L.C., Chakrabarthy, S., Barman, D., & Singh, D. R. (2019). PGR diversity and economic utilization of orchids. *International Journal of Current Microbiology and Applied Sciences* 8(10): 1865-1887.
- Raskoti, B.B., Schuiteman, A., Jin, W.T., & Jin, X.H. (2017). A taxonomic revision of *Herminium* L. (Orchidoideae, Orchidaceae). *PhytoKeys* 79(1): 1-74.
- Rice, A., Glick, L., Abadi, S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O., & Mayrose, I. (2015) The Chromosome Counts Database (CCDB) - a community resource of plant chromosome numbers. *New Phytologist* 206(1):19-26.
- Samatadze, T.E., Yurkevich, O.Y., Haziyaeva, F.M., Konyaeva, E.A., Morozov, A.I., Zoshchuk, S.A., Amosova, A.V., & Muravenko, O.V. (2020). Agromorphological, microanatomical and molecular cytogenetic characterization of the medicinal plant *Chelidonium majus* L. *Plants (Basel, Switzerland)* 9(10): 1-17.
- Setiaji, A., Muna, A., Jati, F.P., Putri, F., & Semiarti, E. (2018). Keanekaragaman anggrek di Daerah Istimewa Yogyakarta. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia* 4(1): 63-68.
- Sharma, S.K. & Mukai, Y. (2015). Chromosome research in orchids: current status and future prospects with special emphasis from molecular and epigenetic perspective. *Nucleus* 58(3): 173-184.
- Shetty, N.K. (2018). Inheritance of chromosomes, sex determination, and the human genome: a new paradigm. *Gender and the Genome* 2(1): 16-26.
- Singh, R.J. (2003). *Plant Cytogenetics*. CRC Press. New York.
- Suliantini, N., Purwantoro, A., & Sulistyarningsih, E. (2004). Keragaman genetik dalam spesies *Caladium bicolor* berdasarkan analisis kariotipe. *Agrosains* 17(2): 235-244.
- Wang, J.H. & Zheng, X.D. (2017). Comparison of the genetic relationship between nine Cephalopod species based on cluster analysis of karyotype evolutionary distance. *Comparative cytogenetic* 11(3): 477-494.
- Young, H.A., Sarath, G. & Tobias, C.M. (2012). Karyotype variation is indicative of subgenomic and ecotypic differentiation in switchgrass.

*BMC Plant Biology* 12(117): 1-12.

Zhang, S., Yang, Y., Li, J., Qin, J.,  
Zhang, W., Huang, W., Hu, H.

(2018). Physiological diversity of orchids. *Plant Diversity* 40(4): 196-208.