

## MORPHOLOGICAL VARIATIONS OF TERRESTRIAL ORCHID *THELYMITRA JAVANICA* BLUME (ORCHIDACEAE: ORCHIDOIDEAE) IN MOUNT ARJUNO, LAWU, AND SUMBING, JAVA - INDONESIA

I Made Saka Wijaya<sup>1</sup>, Budi Setiadi Daryono<sup>2</sup> & Purnomo<sup>3</sup>

<sup>1</sup>) Postgraduated Student Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Email: saka.wijaya@mail.ugm.ac.id / imsakawijaya@gmail.com

<sup>2</sup>) Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Email: bs.daryono@mail.ugm.ac.id

<sup>3</sup>) Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Email: nomo@ugm.ac.id

I Made Saka Wijaya, Budi Setiadi Daryono & Purnomo. 2018. Variasi Morfologi Anggrek Tanah *Thelymitra javanica* Blume (*Orchidaceae*: *Orchidoideae*) di G. Arjuno, G. Lawu dan G. Sumbing, Jawa-Indonesia. *Floribunda* 6(1): 22–31. — Tujuan kajian ini adalah untuk mengetahui variasi morfologi dan kekerabatan fenetik dari *T. javanica* dari ketiga gunung tersebut. Tiga belas sampel dikoleksi dari sepanjang jalur pendakian. Pengamatan morfologi dilakukan terhadap organ akar, umbi, daun, tangkai perbungaan, braktea, bunga, buah dan biji. Dendrogram dibangun dengan metoda kluster UPGMA dengan algoritma “Percent’s Similarity”. Sedangkan analisis PCA dilakukan dengan algoritma “Euclidean Distance” menggunakan perangkat lunak MVSP v.3.1. Hasil studi menunjukkan bahwa variasi morfologi jenis *T. javanica* tergolong plastis dalam hal ukuran dan warna, terutama warna tangkai perbungaan, mahkota, labelum, ovarium dan rangka ovarium, yang dapat digunakan untuk membedakan populasi atau ekotipe.

Kata kunci: Ekotipe, *Orchidaceae*, fenetik, plastisitas, *Thelymitra javanica*.

I Made Saka Wijaya, Budi Setiadi Daryono & Purnomo. 2018. Morphological Variations of Terrestrial Orchid *Thelymitra javanica* Blume (*Orchidaceae*: *Orchidoideae*) in Mount Arjuno, Lawu, and Sumbing, Java-Indonesia. *Floribunda* 6(1): 22–31. — The aims of this study were to observe morphological variations and phenetic relationships of *T. javanica* from the three mountains. Thirteen samples were collected along the tracking routes, morphological observation was based on character of root, tuber, leaf, peduncle, floral bract, flower, fruit, and seed. The data were compile descriptively to identify variations that subsequently scored. Dendrogram were constructed by UPGMA clustering method with Percent’s Similarity algorithm, while Principal Component Analysis was done with Euclidean Distance algorithm using MVSP v.3.1. software. The result shows that morphological variations of *T. javanica* is found to be plastis for size and color, especially color of peduncle, petals, labellum, ovary, and ovary ribs that can be used to separate distinct population or ecotype.

Keywords: Ecotype, *Orchidaceae*, phenetic, plasticity, *Thelymitra javanica*

Indonesia has part of Sundaland and Wallacean as the center of biodiversity with some conservation priorities and high number of endemic plants (Myers *et al.* 2000). One of the plant family that has high endemicity is orchids (*Orchidaceae*), that not only adaptive, but also showing great evolutionary traits especially from the floral characters (Dressler 1993; Kindlman & Jersakova 2005). Orchids are cosmopolite plants, distributed from the sea shore to the mountain (Dressler 1993; Brown *et al.* 2008; Roberts & Dixon 2008; Swartz & Dixon 2009). Some orchid species might grow at the top of the mountain in a distinct habitat such as volcano.

*Thelymitra* is a genus of terrestrial orchid that distributed mainly in Australia with over 110 species (Jeanes 2013). Most species are distributed in lowland area (Edens-Meier *et al.* 2013; Jeanes 2013; Edens-Meier & Bernhardt 2014), while *T. javanica* that occur in Indonesia is occupied highland. This species grow at the altitude 1,400–3,300 meters above sea level (m asl), dominantly at >2,000 m asl, with high light intensity and sandy soils (Backer & Bakhuizen v.d. Brink 1968). *T. javanica* also tolerates soil with high content of sulphur that allow the plant to grow near the peak of active volcano (Comber 1990). *T. javanica* Blume is distributed in Java, Lombok (Kalkman

1955; Steenis 1972; Comber 1990), and also some record from Luzon, Philippine (Steenis 1972) which need further investigation.

*Thelymitra* has being well studied by Berhardt, Edens-Meier, and Jeanes in morphological and pollination mechanism. However, there is insufficient taxonomical studies for species *T. javanica*, while there is concern that half of terrestrial orchid species were extinct (Swartz & Dixon 2009) which might includes this species. *T. javanica* occupied specific habitat and has disjunct distribution that probably lead to its intraspecific variation both genetically or morphologically. This study was conducted to evaluate morphological variations and phenetic relationships of *T. javanica* from different ecotypes in Java. The study sites

were in three mountains, i.e. Mount Arjuno (East Java), Lawu (borderline of East and Central Java), and Sumbing (Central Java).

## MATERIALS AND METHODS

### Data Collection

The study was conducted in three mountains: Mount Arjuno (East Java), Lawu (borderline of East and Central Java), and Sumbing (Central Java), on August 2016–August 2017 (Fig 1). *T. javanica* usually found in open area or under the shade of grass, eidelweis (*Anaphalis* spp.), cantigi (*Vaccinium varingiaefolium*), *Casuarina junghuhniana*, and various type of grass (*Poaceae* and *Cyperaceae*).

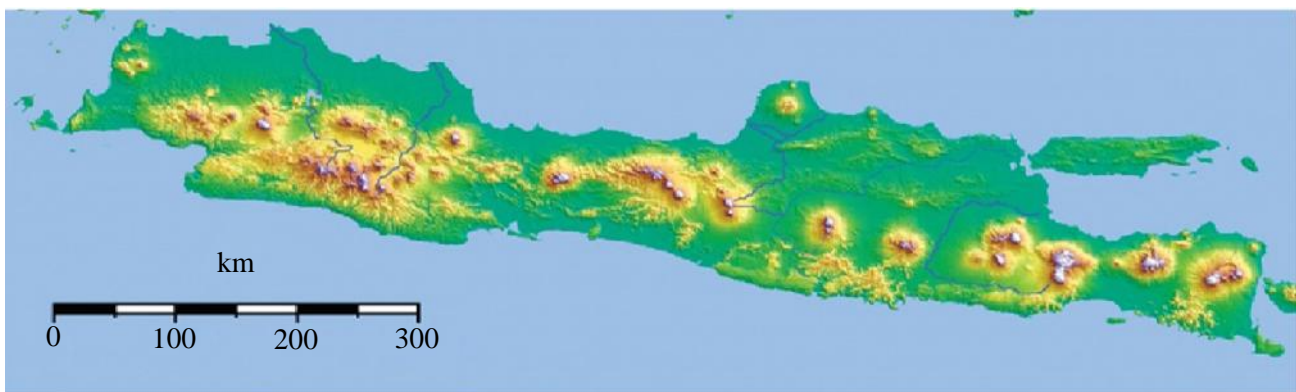


Figure 1. Study site of *T. javanica* in Mount Arjuno, Lawu, and Sumbing.

Randomly, choosed 3–5 populations from each mountain with different altitude as a sampling unit, and collected 1–3 individual of each population as a repetition (Table 1). Total of samples were 13, consist of 5 samples from Mount Arjuno, 5 samples from Mount Lawu, and 3 samples from Mount Sumbing. Morphological data were also collected by photo documentations (Baskauf & Kirchoff 2008) for its habitat, whole plant, and flower including close up for column as a specific character in *Thelymitra* (Burns-Balogh & Bern-

hardt 1988; Jeanes 2013). The morphological description for each sampling unit following description and terminology by Averyanov (2008) and Jeanes (2013). Character observed in this study were root and tuber, leaf, peduncle, flower, fruit, and seed. The colors of every observed characters was compared to the 6<sup>th</sup> edition Royal Horticulture Society (RHS) color chart (2015). Specimens were collected as spirit specimens and deposited in Herbarium Bogoriense, Indonesian Institute of Science (LIPI).

Table 1. Sampling units of *T. javanica*

Mount	Sampling Unit	Altitude (m. asl.)	Code
Arjuno	1	3,026	Arj-01
Arjuno	2	3,084	Arj-02
Arjuno	3	3,119	Arj-03
Arjuno	4	3,196	Arj-04
Arjuno	5	3,323	Arj-05
Lawu	1	2,478	Lawu-01

Table 1. Sampling units of *T. javanica* (continued)

Mount	Sampling Unit	Altitude (m. asl.)	Code
Lawu	2	2,714	Lawu-02
Lawu	3	2,995	Lawu-03
Lawu	4	3,116	Lawu-04
Lawu	5	3,243	Lawu-05
Sumbing	1	3,140	Sum-01
Sumbing	2	3,193	Sum-02
Sumbing	3	3,201	Sum-03

**Data Analysis**

Morphological data were analyzed descriptively to identify the variations in character of observed organs, which completed in MVSP v.3.0 (Multivariate Statistical Package) software to construct phenetic dendrogram and for Principal Component Analysis (PCA). Dendrogram constructed by Percent Similarity algorithm and Unweighted Pair Group with Mean of Arithmetic (UPGMA) clustering method, while the PCA scattered plot was constructed using Euclidean Distance algorithm. PCA was implemented to indicate characters that contribute the most in generating distinct

group. 83 morphological characters have been generated, but only 19 qualitative characters found to be sufficiently varies among sampling units (Table 2). The data set resulted from observation of 13 samples that is used for numerical analysis is presented in Table 3. Most of these characters are colors which considered as impression of plastic adaptation and is suggested to be avoided (Sokal & Sneath 1963). However, in this study, color is viewed as an acceptable character to discriminate variation within a species compare to other observed characters, as demonstrated in the aforementioned analysis.

Table 2. Morphological characters and scores of *T. javanica*

Code	Characters	Character Scores
K1	Color of peduncle	1: green (RHS2015143A), 2: purplish pink (RHS2015076B), 3: purplish red (RHS2015077B)
K2	Color of leaf base	1: green (RHS2015143A), 2: purplish red (RHS2015077B)
K3	Color of leaf blade	1: green (RHS2015143A), 2: purplish red gradated green (RHS2015077B – RHS2015139B)
K4	Color of leaf vein	1: green (RHS2015143A), 2: purplish red (RHS2015077B)
K5	Leaf's vein clarity	1: blurry, 2: clear
K6	Color of bractea	1: green (RHS2015143A), 2: greenish purplish red (RHS2015144D), 3: purplish red (RHS2015077B)
K7	Color of median adaxial sepal	1: pale green gradated purplish pink (RHS2015146D – RHS2015076B), 2: green gradated purplish red (RHS2015144D – RHS2015077B), 3: purplish red (RHS2015077B)
K8	Color of abaxial sepal	1: purplish pink gradated purplish red (RHS2015076B – RHS2015077B), 2: purplish red (RHS2015077B), 3: blue gradated purplish red (RHS2015085B, RHS2015100B – RHS2015077B)
K9	Color of petal	1: purplish pink gradated purplish red (RHS2015076B – RHS2015077B), 2: purplish red (RHS2015077B), 3: blue gradated purplish red (RHS2015085B, RHS2015100B – RHS2015077B)

Table 2. Morphological characters and scores of *T. javanica* (continued)

Code	Characters	Character Scores
K10	Color of labellum	1: purplish pink gradated purplish red (RHS2015076B – RHS2015077B), 2: purplish red (RHS2015077B), 3: blue gradated purplish red (RHS2015085B, RHS2015100B – RHS2015077B)
K11	Color of column	1: white (RHS2015NN155C), 2: white with purplish margin (RHS2015NN155C – RHS2015077B)
K12	Color of the top dorsal of column	1: brown gradate yellow (RHS2015165A – RHS2015015C), 2: brownish orange (RHS2015169A)
K13	Contour of the top dorsal of column	1: smooth-grooved-wide, 2: smooth-grooved-narrow
K14	Color of column's wings	1: white (RHS2015NN155C), 2: purplish white (RHS2015075C)
K15	Color of column's thricomes	1: white (RHS2015NN155C), 2: white with purplish base (RHS2016075C)
K16	Dense of column's thricomes	1: dense, 2: loose
K17	Color of pollinia	1: white (RHS2015NN155C), 2: yellow (RHS2015161C)
K18	Color of ovary	1: green (RHS2015144B), 2: purplish green (RHS2015N77A), 3: purplish red (RHS2015077B)
K19	Color of ovary ribs	1: green (RHS2015144B), 2: purplish green (RHS2015N77A), 3: purplish red (RHS2015077B, RHS2015084B)

Table 3. Data set of *T. javanica* characters (K) and scores for numerical taxonomy analysis

K / Score	Arj-01	Arj-02	Arj-03	Arj-04	Arj-05	Lawu-01	Lawu-02	Lawu-03	Lawu-04	Lawu-05	Sum-01	Sum-02	Sum-03
K1	3	3	3	3	3	1	2	2	3	3	2	1	1
K2	2	2	1	2	2	2	2	2	2	2	1	1	1
K3	1	2	1	1	2	1	1	1	1	1	1	1	1
K4	2	2	2	2	2	2	2	2	2	2	1	1	1
K5	2	2	2	2	2	2	2	2	2	2	2	1	1
K6	2	3	2	3	3	2	2	2	3	2	2	1	1
K7	1	3	2	3	3	2	2	1	1	1	1	1	2
K8	1	1	2	2	2	1	3	1	1	1	1	1	1
K9	3	3	2	2	2	1	3	1	1	1	1	1	1
K10	3	3	2	2	2	1	3	1	1	1	1	1	1
K11	1	1	1	1	1	1	2	1	1	1	1	1	1
K12	1	2	1	1	1	1	1	1	1	1	1	1	1
K13	1	2	1	1	1	1	1	2	2	1	1	1	2
K14	2	2	1	2	2	1	1	1	1	1	2	2	2
K15	1	2	1	1	1	1	1	2	1	1	1	1	1
K16	1	1	1	1	1	2	2	1	1	1	1	1	1
K17	1	1	2	2	1	1	1	2	1	1	1	1	1
K18	1	1	3	3	3	1	1	2	2	2	1	1	1
K19	3	3	3	3	3	1	1	2	2	2	1	1	1

## RESULTS AND DISCUSSION

### RESULTS

The present the study results in two section, i.e. morphological variations and phenetic analyses of *T. javanica*.

#### A. Morphological Variations of *T. javanica* in study site

##### Root and tuber variations

Morphological variations were observed for vegetative and generative organs, including root and tuber. Both characters in *T. javanica* is varies in size and number. Root is generally cylindrical, thick, light brown (RHS2015164C, RHS2015164D), and attached at the base of tuber. Tuber has oblate shape and its number ranged 1–3 tubers in each plant.

##### Leaf variations

*T. javanica* has one linear leaf, conduplicate, entire margin, acute apex, and size 9.6–22.0 x 0.5–0.7 cm. Variations occur in the color of leaf blade, leaf base, and veins consist of green (RHS2015143A) and purplish red (RHS2015077B). The bractea of *T. javanica* has lanceolate shape, entire

margin, and acute apex. The color variations in this organ are green (RHS2015143A, RHS2015144D), purplish green (RHS2015144D-RHS2015077B), and purplish red (RHS2015077B).

##### Inflorescence variations

The type of inflorescence is raceme, 9.1–29.1 cm long, 2–10 flowers, thick and generally has thin white layer (cuticle). The color variation are green (RHS2015143B), purplish pink (RHS 2015076B), and purplish red (RHS2015077B, RHS2015N77A).

##### Flower variations

Sepal of *T. javanica* has narrowly-ovate shape, 1.2–2.2 x 0.4–0.8 cm, entire margin, and acute apex. The abaxial color is varied from purplish pink gradated to purplish red (RHS2015076B-RHS2015077B), purplish red (RHS2015077B), or blue gradated to purplish red (RHS2015085B, RHS2015100B-RHS2015076B). The adaxial part has the same color as its abaxial, but has additional color on the median part which is pale green gradated to purplish pink (RHS2015146D-RHS2015076B), green gradated to purplish red (RHS2015144D-RHS2015077B), and purplish red (RHS2015077B) (Fig. 2). The purplish red sepal only found in Mount Arjuno's specimens.

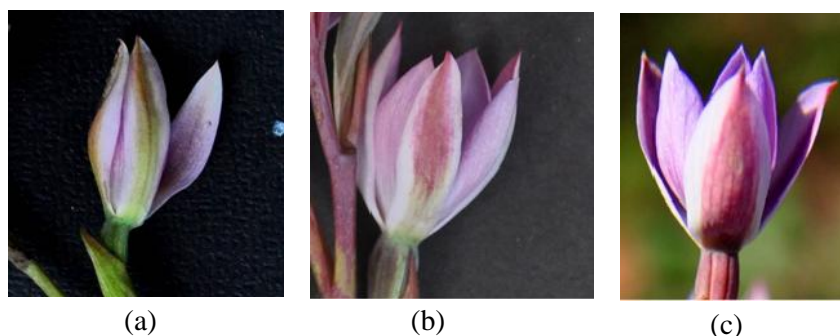


Figure 2. Median sepal variations of *T. javanica* (a). Green gradated to purplish red in Mount Sumbing; (b). Pale green gradated to purplish pink in Mount Lawu; and (c). Purplish red in Mount Arjuno.

Petal has narrowly-ovate shape, 1.1–1.8 x 0.5–1.0 cm, entire margin, and acute apex. Labelum has the same characteristics, except on the size that narrower than petal, about 1.0–1.9 x 0.4–0.6 cm. The color variations are purplish pink gradated to purplish red (RHS2015076B-RHS2015077B), purplish red (RHS2015077B), or blue gradated to purplish red (RHS2015085B, RHS2015100B-RHS2015076B) (Fig. 3a-c). Flower of lower altitude ( $\pm 100$  m asl.) plants shows white perianthium

when blooming (Fig. 3d).

There are variation in column of *T. javanica*. The column's wing has white (RHS2015NN155C) or purplish white (RHS2015075C) color, while the top dorsal of column has brown gradate to yellow (RHS2015165A-RHS2015015C), brownish orange (RHS2015169A), or yellow (RHS2015015C) for plant in lower altitude (Fig. 3e-g). Apex of the top dorsal of column forming a wide or narrow hole with a central groove (Fig. 3h-i).

The column's trichomes below the top dorsal of column divided into two groups with color variation in white (RHS2015NN155C) or white with purplish base (RHS2015NN155C-RHS2015075C). The distance between the two groups of trichomes might be fairly wide or narrower (Fig. 3i-j, pointed

by red arrow). Column has white (RHS2015NN155C) or white with purple margin color (RHS2015NN155C-RHS2015077B), 0.4–0.6 x 0.2–0.3 cm, stigma cavity 0.2–0.4 x 0.1–0.3 cm, two pairs of pollinia in white (RHS2015NN155C) or yellow color (RHS2015161C).

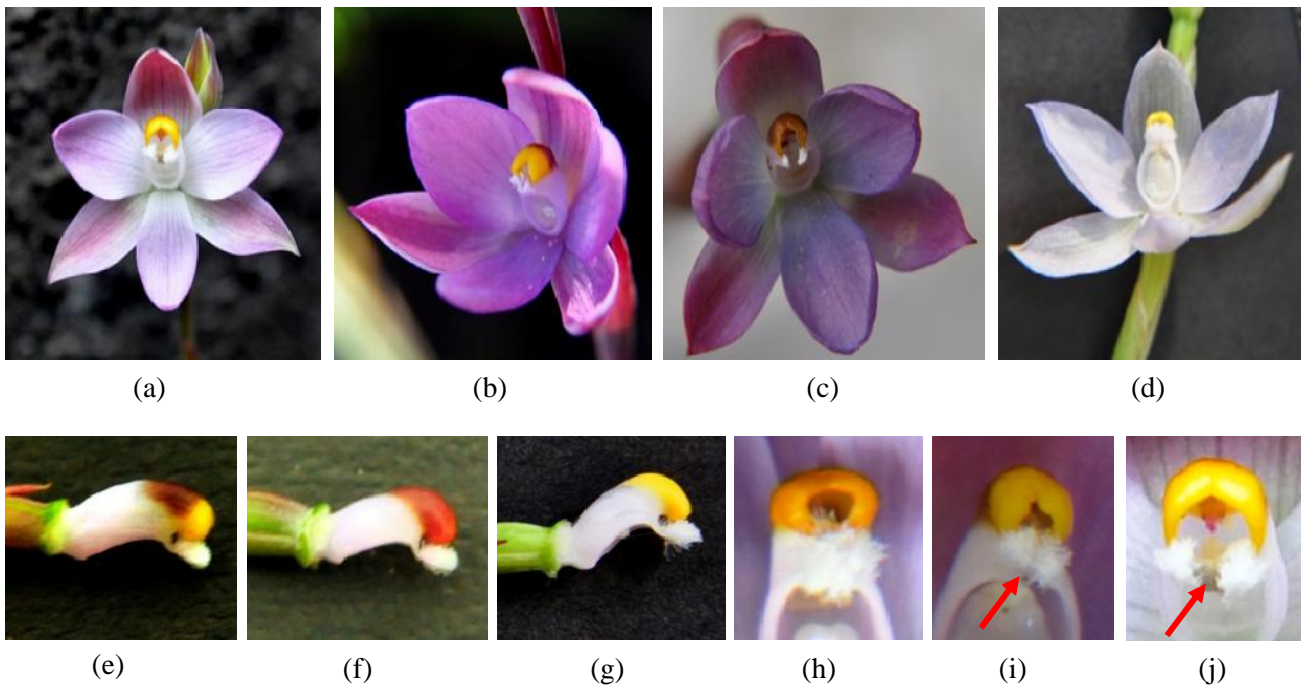


Figure 3. The flower variations of *T. javanica* (a). Petal and labellum with purplish pink gradated to purplish red color in Mount Lawu; (b). Petal and labellum with purplish red color in Mount Arjuno; (c). Petal and labellum with blue gradated to purplish red color in Mount Lawu; (d). Petal and labellum with white color at lower altitude; (e). The top dorsal of column in Mount Arjuno: brown gradated to yellow, and (f). brownish orange; (g). The top dorsal of column at lower altitude: yellow; (h). the top dorsal of column's hole in Mount Arjuno: wide; (i). the top dorsal of column's hole: narrow, with dense trichomes (red arrow) in Mount Lawu; (j). Loose trichomes (red arrow) in Mount Lawu.

### Fruit and seed variations

The fruit was oblong in shape with ovary color in green (RHS2015144B), green with purplish red ribs (RHS2015147B-RHS2015084B), purplish green (RHS2015N77A), or purplish red (RHS2015077B) (Fig. 4a-d). The seed is bottle-

shaped, testa formed by rectangular cells that covers the oval embryo with brown color (RHS2015164C) (Fig. 4e-h). The seed size varies between 253.69  $\mu\text{m}$  to 443.98  $\mu\text{m}$ , while the embryo 149.12  $\mu\text{m}$  to 253.39  $\mu\text{m}$ . The seed/embryo ratio ranged 1.59–2.31, but commonly around 2.0.



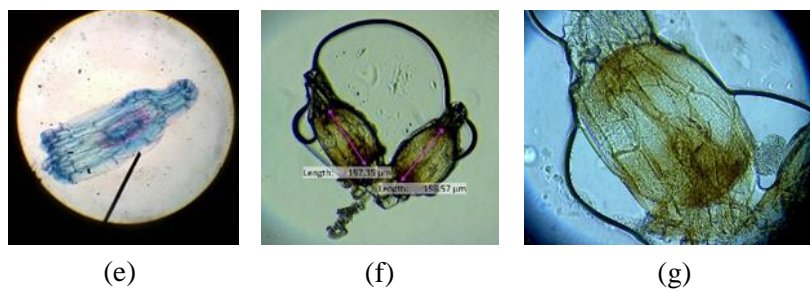


Figure 4. Fruit and seed variations of *T. javanica* (a). Green ovary from Mount Lawu; (b). Green with purplish red ribs ovary from Mount Arjuno; (c). Purplish green ovary from Mount Lawu; (d). Purplish red ovary from Mount Arjuno; (e). Bottle-shaped seed with oval embryo in 4x magnification with toluidine blue dye; (f). Seed with embryo sizing in Mount Arjuno, 10x magnification; and (g). The embryo of seed from Mount Arjuno, 40x magnification.

**B. Phenetic Relationship of *T. javanica* based on Morphological Characters**

The phenetic relationship of *T. javanica* using 19 characters and 13 sampling unit that consist of 5 from Mount Arjuno (code: Arj), 5 from Mount Lawu (code: Lawu), and 3 from Mount Sumbing (code: Sum). The dendrogram that has been constructed showing two clusters at the phenon line of 80% similarity (Fig. 5). The cluster 1 composed by all sampling unit from Mount Sumbing and Lawu, except Lawu-02 that merged with sampling units from Mount Arjuno in cluster 2.

With phenon line at 85% similarity, cluster 1

divided into two subcluster, i.e. subcluster a (Lawu-01, Lawu-03, Lawu-04, and Lawu-05) and sub-cluster b (Sum-01, Sum-02, and Sum-03). In cluster 2, all sampling units from Mount Arjuno (Arj-01, Arj-02, Arj-03, Arj-04, and Arj-05) located in subcluster d, leaving Lawu-02 alone in subcluster c.

PCA result in Fig. 6 shows that there are 3 main clusters and one separating cluster consist only Lawu-02. This departing group of Lawu-02 is similar to the discussed dendrogram. The three main clustering resulted from PCA also in line with the dendrogram.

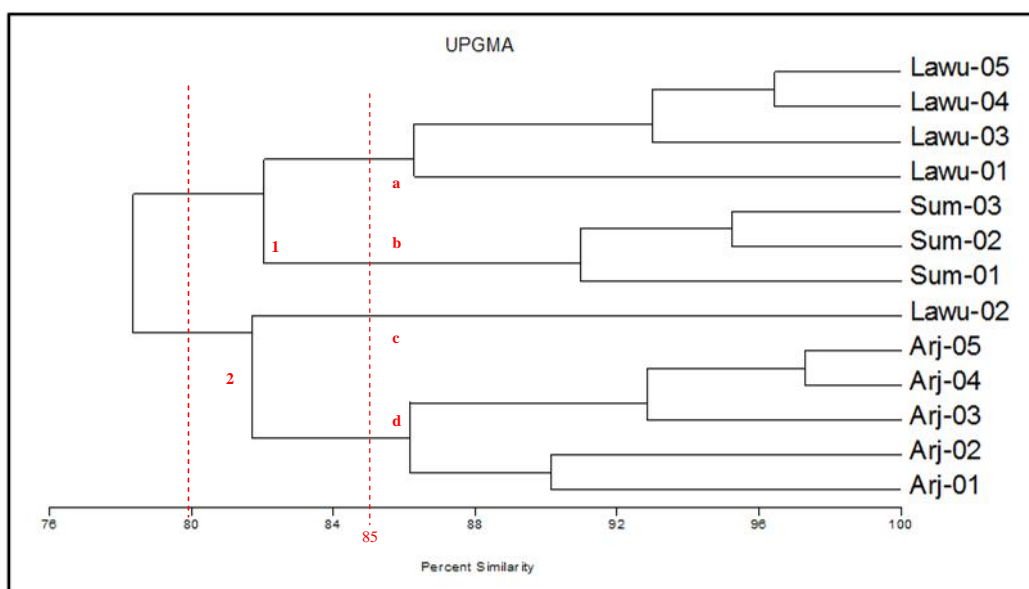
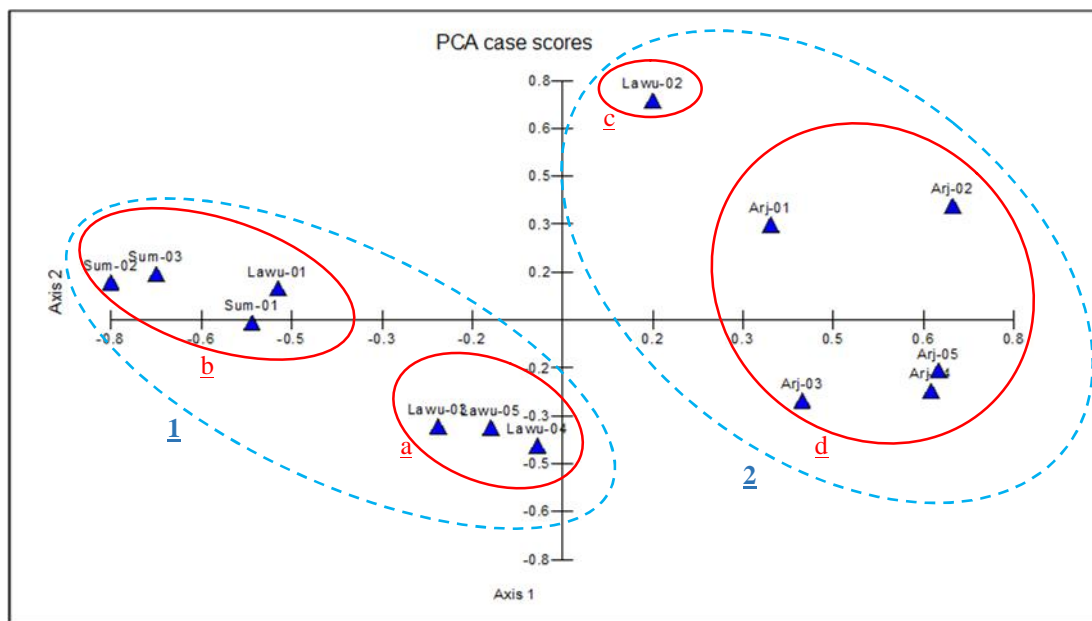
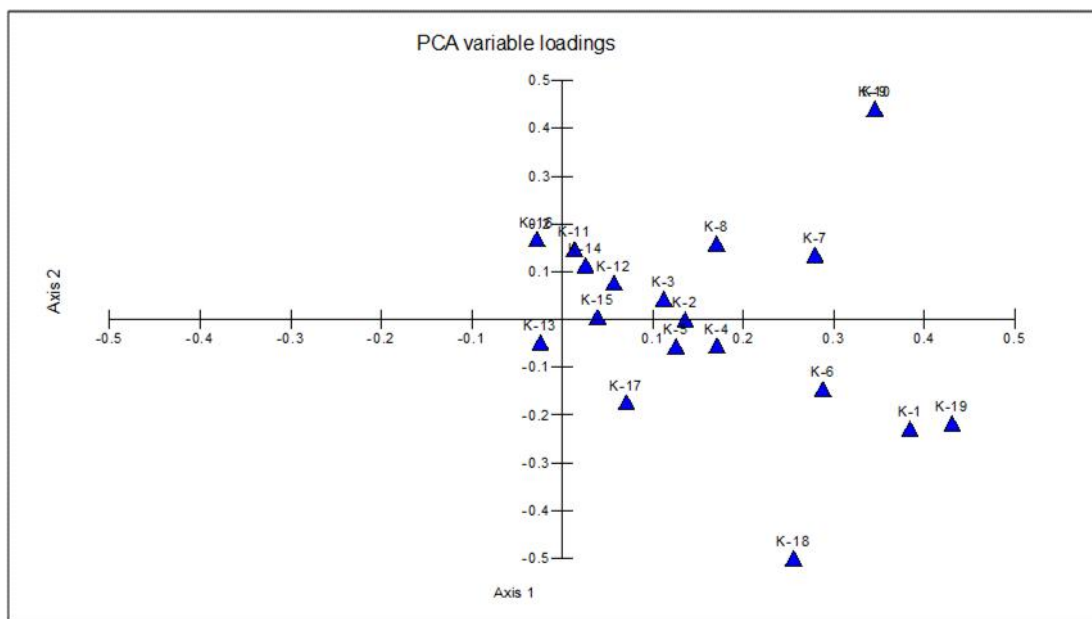


Figure 5. Dendrogram that showing phenetic relationship of *T. javanica* based on morphological characteristics in Mount Arjuno, Lawu, and Sumbing.

Note: Arj = Mount Arjuno; Lawu = Mount Lawu; Sum = Mount Sumbing



(a)



(b)

Figure 6. PCA scattered plot by Euclidean distance algorithm for phenetic relationship of *T. javanica* based on morphological characteristic in Mount Arjuno, Lawu, and Sumbing (a). Sampling units' ordination that forming cluster; and (b). Character distribution that responsible for cluster forming.

Note: Arj = Mount Arjuno; Lawu = Mount Lawu; Sum = Mount Sumbing

The cluster separation in PCA is primarily caused by morphological character with code K-1, K-9, K-10, K-18, and K-19. Those characters has the longest vector (distance) that indicate its higher impact in cluster formations. The distance is measured from the center point (0,0) to the characters' code. The eigenvalues also confirmed that those characters are forming two orientations, to the Axis

1 and Axis 2. K-1 is a code for peduncle's color, K-9 for petal's color, K-10 for labellum's color, K-18 for ovary's color, and K-19 for ovary ribs' color. K-6 and K-7 are codes for bractea's color and median adaxial sepal's color, that also showing higher eigenvalue more than 0.2, but those are only partially impacting ordination, i.e. for Axis 1.



## DISCUSSION

### Morphological Variations of *T. javanica*

Morphological variations of *T. javanica* is dominated by generative organ. This is shown in variability of color. Most of *T. javanica* perianthium has purplish red color while flower of individuals in lowland ( $\pm 100$  m. asl.) exhibit white color that could be caused by flavon and flavonol (Saito *et al.* 2007; Daniel 2009). This color variation demonstrate character plasticity that once used to classified population as ecophene or plastodeme, populations that genotypically similar but different phenotypically (Stace 1989).

### Phenetic Relationship of *T. javanica* based on Morphological Characters

Phenetic dendrogram shows that the similarity of OTUs from Mount Arjuno and Sumbing has some relation with altitude. In Mount Arjuno, OTU Arj-01 and Arj-02 (joined at 90.14 % similarity) collected from the lowest altitude (3,026 and 3,084 m. asl.), was growing under the shading of *C. junghuhniana* and among grasses. OTU Arj-04 and Arj-05 (with highest similarity, 97.30 %) was growing near the peak, at altitude 3,196 and 3,323 m. asl respectively, in the open area or under the shading of eidelweiss plant (*Anaphalis* spp.) and cantigi (*V. varingiaefolium*).

In Mount Sumbing, although the three OTUs were collected at different altitude (3.140, 3.193, and 3.201 m asl) they still grew in same identical habitat which is on the rock in savana Segara Banjaran. Furthermore, the response for the environ-

mental variations were identic and showing high similarity (all at 90,96%, and Sum-02 and Sum-03 at 95.24%) that confirm the character plasticity by environmental adaptations (Stace 1989).

The analysis also shows that OTU Lawu-02 is more similar to OTUs from Mount Arjuno than other unit from Mount Lawu, although the similarity level was 81.71%. The separation is still unexplained. Similar split also found in OTU Lawu-02 that appear to have closer relationship with OTUs from Mount Sumbing.

The cluster formation by altitude as shown in this study confirmed that morphological variations observed were plastic, as a result of environmental adaptation (Elzinga *et al.* 2007). The pigment anthocyanin produced by this plant seems to be increasing with the higher altitude (Chalker-Scott 1999; Saito *et al.* 2007; Daniel 2009; Ramakrishna & Ravishankar 2011). This study also showed there are site-specific coloration occurred in different mountains. Mountain might have several ecotypes, showing character plasticity that is caused by altitude. This merit further investigation with more advance method such as molecular approaches.

Color is generally considered as high-risk character to base classification for it might resulted from environmental adaptation and hence thought as a plastic character. The case in *T. javanica* indicates this, color variation emerges as the respons of environmental variability. However, since every mountain shows distinct plastic characters, this could be used in differentiating separated population. The key to population is as follow:

- 1.a. Leaf base's color and nerve's color in purplish red ..... 2
- b. Leaf base's color and nerve's color in green ..... *T. javanica* Sumbing
- 2.a. Petal and labellum in purplish red or blue gradated purplish red, ovary ribs' color in purplish red ..... *T. javanica* Arjuno
- b. Petal and labellum in purplish pink gradated purplish red (rarely blue gradated purplish red), ovary ribs' color in green or purplish green ..... *T. javanica* Lawu

## CONCLUSION

As the only species from the genus *Thelymitra* from Indonesia, *T. javanica* is found to have morphological plasticity for sizes and colors, especially the colors of peduncle, petal, labellum, ovarium, and ovary ribs. Based on this research, there are three groups of ecotype plasticity (plastodeme) identified: Arjuno, Lawu, and Sumbing.

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