

trichome

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The morphology and density of pasak bumi (*Eurycoma longifolia*, Jack) leaf trichomes in six natural populations in Indonesia

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Abstract. *Eurycoma longifolia* Jack is one of the important medicinal plants in Indonesia and is used in many traditional and modern medicines. Information on trichome characteristics of *E. longifolia* and its relationship with environmental factors is limited. The purpose of this study was to investigate the morphology and density of the leaf trichomes of *E. longifolia*, to observe its correlation with geographic and climate factors of population, and to identify trichome characters that can discriminate among populations. The morphology and density of the leaf trichome of six populations of *E. longifolia* were investigated using a light microscope. Analysis of variance, correlation analysis, principal component analysis (PCA), and clustering of the population were performed. The results of this study detected two types of glandular trichome in the leaf of *E. longifolia*, namely peltate and capitate. Analysis of variance showed a significantly different density of trichome among populations. Variation of trichome density among populations may be caused by different micro-environmental of population and genetic factors of plant individuals. We confirmed that geographic and climate factors of the population have significant positive/negative correlations with types and density of trichomes. The PCA analysis exhibited trichome density could be used as a distinguishing characteristic among populations.

1. Introduction

Pasak bumi (*Eurycoma longifolia*, Jack) is one of the medicinal plants that have economically potential to be developed in Indonesia. They are grown and distributed in the primary forest of the Sumatra and Borneo islands [1–3]. *E. longifolia* is shrubs or small trees with a tall of up to 10 m. This plant possessed many chemical compounds that can be used as anti-inflammatory, antioxidant, antibacterial, anticancer, anti-plasmodial, and increasing the testosterone hormone [4–6]. Now, the



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extract of this plant has been traded in herbal medicinal shape as tea and coffee, as well as the capsule that produced the pharmaceutical industry.

Trichomes are uni or multi-celled pustule, which originate from the epidermal cells. They varied in morphological characters, locations, ability to secrete, and type of secretion [7]. In general, trichome (hairs) can be classified into two main groups, namely non-glandular and glandular trichomes. These trichomes have many roles in each plant species, and they can be found in several organs such as the leaf, petal, bark, etc. Leaf trichomes have two functions in the plant i.e. structural defense against herbivores and protection against environmental factors such as strong UV radiation, solar radiation, drought, high temperature, and freezing [8, 9].

Currently, many researchers used trichomes as a tool for taxonomic identification of plants, not only in the intra-generic classification level [10, 11] but also at the species and sub-species level [12, 13]. This is closely related to the character of trichomes that are relatively stable, relatively diverse, easy in the preparation for study, and commonly found in several plant families [14].

Information about morphology and density of trichomes of the *E. longifolia* species as well as comparison variation of the trichome density between plant populations in different environmental conditions are not available. Description of the morphology of trichomes and their relationships to environmental factors is particularly important to be known since trichome structures produce some of the active compounds [15], and the therapeutic efficacy of the plants might be also affected by environmental factors. Therefore, we conducted the study on trichomes morphology and density present on the leaves of *E. longifolia*. The purpose of this study was to determine the morphology and density of trichomes in the leaves of *E. longifolia*, to observe its relationship with geographic and climate factors of population, and to identify trichome characters that can discriminate among population studies.

17

2. Materials and methods

2.1. Plant samples

The samples of *E. longifolia* were collected from six populations, one population from West Borneo, two populations from Riau islands, and three populations from Riau province. The climatic and geographic data of each population were shown in Table 1. Five mature plants are taken from each population, which are selected randomly and the minimum distance among plants is 20 m. Each plant was taken one leaf expanded fully, and health. The leaves were then made into a herbarium and sent to the laboratory.

Table 1. Research sites characteristic of *E. longifolia*.

| Population name | Research sites status | Longitude | Latitude | Altitude (m.a.s.l) | Temperature mean annually (°C) | Precipitation mean annually (mm year ⁻¹) |
|------------------------------|-----------------------|--------------|------------|--------------------|--------------------------------|--|
| Pokomo, Riau Province | Protected forest | 100°56'31" E | 0°15'40" N | 170 | 27.50 ^a | 225.17 ^a |
| Tahura, Riau Province | Forest park | 101°25'23" E | 0°41'8" N | 70 | 27.50 ^a | 208.60 ^a |
| Mandor, West Borneo Province | Forest nature reserve | 109°20'5" E | 0°18'40" N | 50 | 26.80 ^b | 319.10 ^b |
| Lingga-1, Riau Archipelago | Natural forest | 104°40'25" E | 0°10'39" S | 90 | 27.20 ^c | 236.50 ^c |
| Lingga-2, Riau Archipelago | Protected forest | 104°35'12" E | 0°12'66" S | 160 | 27.20 ^c | 236.50 ^c |
| Sentajo, Riau Province | Protected forest | 101°30'22" E | 0°28'42" S | 106 | 27.50 ^a | 279.66 ^a |

Data sources: a[16], b[17], c[18]

2.2. Procedure for trichome observation

One dry leaflet of *E. longifolia* was taken from each sample leaf for trichome observation. The method of collecting of trichome used the nail polish. Lightly paint a section of the leaflet middle veins with clear fingernail polish. Allow the fingernail polish for minutes to completely dry. Place the clear tape on the dried nail polish. Press the tape onto the leaflet gently and firmly, and then peel the tape from the leaf and place the tape sticky side down of the microscope slide. The tape contains the trichome is observed under a light microscope. Nikon Eclipse 50i (Nikon, Japan) with 400x magnifications. The images are captured using Camera Nikon DS-Fi1 and were analyzed using NIS-Element software. Move the slide to find the trichomes in the other area. The trichome was observed on the abaxial and adaxial leaflet surface. For measurement, each leaflet and side was captured six images, and a total of 30 images for each side per population. The different types and distribution of trichome are described and then compared. The general trichome terminology follows [19] and [14]. Trichome density was calculated by dividing the number of trichome by the view field area [20].

2.3. Statistical analyses

The mean and standard deviation of the observed trichome number was calculated. Analysis of variance (ANOVA) was used to assess the trichome differences among populations and the Pearson correlation coefficient was also used to exhibit a significant correlation between different hair types. The multivariate analyses such as principal component analysis (PCA) and the dendrogram of UPGMA (Unweighted Pair Group Method with Arithmetic Mean) were performed. The used software for statistical analysis was SAS ver.9. [21] and NTSYS ver. 2.01 [22].

3. Results and discussion

3.1. Trichome morphology

In general, two types glandular trichomes were observed on the abaxial and adaxial of the leaflet of *E. longifolia*; namely peltate and capitate. Both trichomes were different in size, structure, and mode of secretion. Peltate trichome was constituted by a cell at the base, a very short stalk cell, and a large secretory head forming the central and peripheral cells (Figure 1). The head of this trichome displayed a typical spherical shape due to cuticle expansion during the accumulation of essential oil in the sub-cuticular space. This peltate trichome is also observed in Lamiaceae family [14], and *Salvia argentea* [23].

Capitate trichome was constituted by one basal cell, a stalk of variable length, and uni- or bi-cellular head, in which the head size is smaller than that of peltate trichomes (Figure 2–4). In this study, capitate trichomes can be distinguished into three types based on the morphology and dimension of the stalk, namely capitate trichome of Type I, capitate trichome of Type II, and capitate trichome of Type III. Capitate trichome of Type I had a bicellular rounded to oval head, a body with a unicellular short stalk, and a unicellular basis (Figure 2).

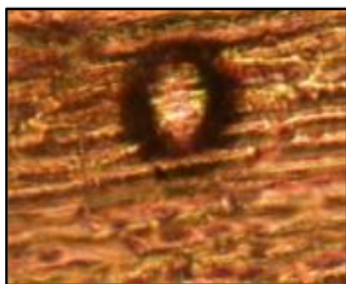


Figure 1. Peltate trichome.

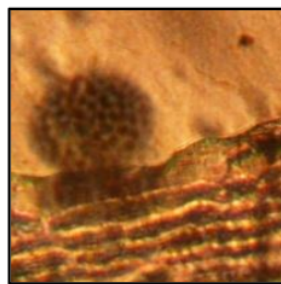


Figure 2. Capitate trichome of Type-I.



Figure 3. Capitate trichome of Type-II.



Figure 4. Capitate trichome of Type-III.

Capitate trichome of Type II constituted by a unicellular rounded head and body with a short neck cell, a bicellular long stalk, a large unicellular pedestal, and a 6–8 celled basis (Figure 3). Capitate trichome of Type III constituted by one cell head, long-stalked (4–7 cells), and one cell basal. This type matches the description and typology of [13], in which the capitate trichome of type III corresponds to their type 14A (Figure 4). This type is commonly found form in *E. longifolia*. Peltate and capitate trichomes found on the leaflet of *E. longifolia* showed similar characteristics to the glandular trichome reported for the genus *Leucas* [14]. The presence of peltate and capitate trichome appears to be a common characteristic for a large number of medicinal plant species [24].

3.2. Variation of trichome morphology and density between populations

The results of the analysis of variance showed that the number of trichomes peltate, capitate-type I, and capitate-type II were significant differences between populations in the abaxial leaf surface, while the numbers of capitate-type I, capitate-type II, and capitate-Type III trichomes were significant differences in adaxial leaf surface (Table 2). On the abaxial surface, the highest number of peltate trichome was observed in the West Borneo population, and the lowest value was observed in the Tahura population. The highest number of capitate-1, capitate-2, and capitate3 trichomes were observed in the Lingga-1, Sentajo, and Lingga-2 populations, respectively. On the adaxial surface, the highest number of peltate trichome was observed in the Pokomo population, followed by the Lingga-1 and West Borneo populations, whereas the rest populations were not found of peltate trichome. The highest numbers of capitate-1, capitate-2, and capitate-3 trichomes were observed in the Lingga-2, Sentajo, and Pokomo populations, respectively, while the lowest value was observed in the Sentajo, Lingga-1, and Sentajo populations.

The differences in the number of each trichome between populations are closely related to the environmental conditions of the population and the genetic differences between individuals within the population. Some environmental factors that influenced appearing of trichome were water availability, soil properties, herbivore abundance [8] whereas genes involved in trichome production were *GLABROUS1 (GL1)*, *GLABROUS2 (GL2)*, *GLABROUS3 (GL3)*, *ENHANCER OF GLABRA 3 (EGL3)*, *TRANSPARENT TESTA GLABRA 1 (TTG1)* and *TRYPTYCHON (TRY)* [25,26]. Furthermore, [25] stated that *GLABROUS1 (GL1)* gene is the most promising candidate for trichome variation in the *Artemisia* plant.

The density of *E. longifolia* trichomes on the adaxial leaf surface was higher than the abaxial leaf surface. The average value of trichome density on the adaxial and abaxial leaf surfaces was 415.34 mm⁻² and 63.39 mm⁻², respectively, in which was 6.55 times higher in the adaxial than abaxial leaflet surface. The results of the analysis of variance (ANOVA) showed that the density of trichomes in the abaxial and adaxial leaf surfaces showed a highly significant difference ($P < 0.0001$). The high density of glandular trichomes on the upper surface compared to the lower surface is an important strategy of the plant to deal with high light intensity striking the plant leaves the surface, increasing sunlight reflection, reduce water loss in leaf surface, and the role as the chemical defense against biotic

agents [27, 28]. The higher density of trichomes in adaxial leaf surface compared to the abaxial leaf surface was also reported by [29] in *Hyptis villosa* Pohl ex Benth, [28] in the species of Stachytarphyta, and [12] in *Lipia graveolens* H.B.K., but many researchers also reported that trichome density was higher on the abaxial in some plants [15, 30].

Table 2. Mean value of each trichome in the population studies.

| Position | Trichome type | Population | | | | | |
|----------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | Tahura | Sentajo | Pokomo | Lingga-1 | Lingga-2 | West Borneo |
| Abaxial | Peltate* | 0.067 ^b | 0.267 ^b | 0.100 ^b | 0.467 ^{ab} | 0.233 ^b | 0.833 ^a |
| | Capitate-Type I** | 0.633 ^{ab} | 0.267 ^{bc} | 0.000 ^c | 1.033 ^a | 0.200 ^c | 0.400 ^{bc} |
| | Capitate-Type II** | 0.433 ^{bc} | 2.967 ^a | 0.500 ^{bc} | 0.733 ^{bc} | 0.133 ^c | 1.000 ^b |
| | Capitate-Type III | 0.767 ^{ab} | 0.467 ^b | 0.900 ^{ab} | 0.767 ^{ab} | 1.267 ^a | 0.667 ^b |
| Adaxial | Peltate | 0.000 | 0.000 | 0.167 | 0.067 | 0.000 | 0.067 |
| | Capitate-Type I** | 3.667 ^{bc} | 1.100 ^d | 4.80 ^{ab} | 6.167 ^a | 7.067 ^a | 1.900 ^{cd} |
| | Capitate-Type II** | 2.833 ^b | 14.667 ^a | 1.533 ^b | 0.567 ^b | 1.033 ^b | 2.100 ^b |
| | Capitate-Type III** | 13.100 ^a | 4.767 ^c | 8.367 ^b | 7.633 ^{bc} | 6.767 ^{bc} | 7.833 ^b |

Note: * and ** showed significant difference at $P < 0.05$, and $P < 0.01$, respectively. The same letters in the same line are no significant differences.

The results of the analysis of variance (ANOVA) showed that the density of trichomes in the abaxial and adaxial surfaces was a highly significant difference among populations ($P < 0.0001$). The highest value of trichome density on the adaxial leaf surface is observed in the Sentajo population of 517.21 mm⁻², whereas the lowest value of trichome density is observed in the population of West Borneo of 358.52 mm⁻². The result of the Duncan tested showed that Sentajo and Tahura populations were significantly different from other populations. The average value of trichome density on the abaxial surface was ranged from 37.78–99.92 mm⁻², which the highest of the density of trichome was observed in the Sentajo population and the lowest value of the trichome density was observed in the population of Pokomo. The difference in trichome density between populations may be caused by genetic differences among individuals within the population and micro-environment conditions of each population. These results are the same as those reported by [11] in *Salvia nemorosa* L.

Table 3. Density of trichome between populations and both leaf surfaces of *E. longifolia*.

| Position | Populations | | | | | | Mean |
|------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | Tahura | Sentajo | Pokomo | Lingga-1 | Lingga-2 | West Borneo | |
| Abaxial*** | 47.86 ^{cd} | 99.92 ^a | 37.78 ^d | 75.57 ^{ab} | 46.18 ^d | 73.05 ^{bc} | 63.39 ^b |
| Adaxial*** | 493.70 ^a | 517.21 ^a | 376.99 ^b | 363.56 ^b | 382.03 ^b | 358.52 ^b | 415.34 ^a |

Note: *** showed significant differences level at $P < 0.0001$ among populations according to the result of anova.

3.3. Correlation between trichome morphology and density with site environmental factors

The results of correlation analysis among trichomes types and density with climatic and geographic factors were exhibited in Table 4. Peltate trichome in the abaxial leaflet surface was positive and significantly correlated to mean annual precipitation and longitude and was negative significantly correlated with mean annual temperature and altitude. Capitate trichome of type I in the abaxial leaflet surface was negative significantly correlated to altitude, whereas capitate trichome of type I in the adaxial leaflet surface was a positive significant correlation with altitude, and was a negative significant correlation with mean annual precipitation. Capitate trichome of type II in abaxial leaflet surface was negative significantly correlated to altitude and positive significant correlation with mean annual precipitation, whereas capitate trichome of type I in the adaxial leaflet surface was positive significantly correlated with mean annual precipitation, mean annual temperature, and was negative significantly correlated to longitude and latitude. Capitate trichome of type III in the abaxial leaflet surface was not significantly correlated to climatic and geographic factors of the population studies, whereas capitate trichome of type III in the adaxial leaflet surface was a positive significant correlation with latitude and was negative significantly correlated with mean annual precipitation.

Trichome density in the abaxial leaflet surface was negative significantly correlated to altitude and latitude and was positive significantly correlated to mean annual precipitation, whereas trichome density in the adaxial leaflet surface was a positive significant correlation with mean annual temperature and was negative significantly correlated to longitude. Our results were a line with those reported by [31] in which trichome density in the abaxial leaflet showed a negative significant relationship with altitude. [27] also reported a correlation of trichome density with mean monthly precipitation. In addition, [32] and [33] found that the soil type of plant habitat has an intense influence on the trichome of *Ziziphora tenuior* L and *Acinos graveolens* (M.B.) Link, respectively.

The results of correlation analysis also explained that high trichome density in the adaxial leaflet surface is closely related to temperature. Adaxial leaflet surface receives higher temperatures than abaxial leaflet surface, and plants will present higher trichomes density as a defense mechanism against excessive radiation and higher temperature. [34] explained that the secretion of chemical compounds from trichomes acts as a protector of plant photosynthetic tissue. The secretions reflect excess solar radiation and also help dissipate absorbed heat. As a result, leaf temperatures are maintained at near-optimal levels for photosynthesis and carbon fixation.

Table 4. The correlation coefficients among trichomes types and density with climatic and geographic factors of the population studies.

| Position | Variable | Environmental factors | | | | |
|----------|--------------------|---------------------------------|---------------------------------|------------------------------|--------------------------------|--------------------------------|
| | | Longitude | Latitude | Altitude | MAT* | MAP** |
| Abaxial | Peltate | 0.2421 (0.0011) | -0.0238 (0.7517) | -0.1534 (0.0397) | -0.2395 (0.0012) | 0.2179 (0.0033) |
| | Capitate - Type I | 0.0693 (0.3552) | 0.0167 (0.8235) | -0.1948 (0.0088) | -0.0619 (0.4089) | -0.0404 (0.5908) |
| | Capitate - Type II | -0.0974 (0.1932) | -0.3297 (<.0001) | -0.13 (0.0819) | 0.1215 (0.1043) | 0.3141 (<.0001) |
| | Capitate -Type III | 0.0085 (0.909) | 0.0093 (0.902) | 0.1445 (0.053) | -0.0181 (0.8093) | -0.1094 (0.1436) |
| | Trichome Density | 0.0822 (0.2725) | -0.241 (0.0011) | -0.1941 (0.009) | -0.0644 (0.3905) | 0.2592 (0.0004) |
| | Peltate | -0.0054 (0.9418) | 0.0419 (0.5764) | 0.056 (0.4237) | -0.0032 (0.9657) | -0.0094 (0.9007) |
| Adaxial | Capitate - Type I | -0.0244 (0.7454) | -0.033 (0.6604) | 0.236 (0.0014) | 0.0068 (0.9277) | -0.2773 (0.0002) |
| | Capitate - Type II | -0.2677 (0.0003) | -0.3787 (<.0001) | -0.0652 (0.3844) | 0.2989 (<.0001) | 0.2619 (0.0004) |
| | Capitate -Type III | -0.0757 (0.3124) | 0.3834 (<.0001) | -0.1339 (0.0732) | 0.0763 (0.3086) | -0.2287 (0.0020) |
| | Trichome Density | -0.3279 (<.0001) | -0.0219 (0.7705) | -0.0954 (0.2027) | 0.3527 (<.0001) | -0.0755 (0.3140) |

Note: * mean annual temperature, ** mean annual precipitation.

3.4. Principal component analysis and dendrogram

To know the contribution of each variable in separating of population, the principal component analysis was conducted. Two principal components (PC1 and PC2) explained 99.79% of the total variation among populations (Table 5). PC1 explained 91.01% of the total variation in which the highest contribution was trichome density in adaxial while PC2 explained 8.79% which the variable responsible for distinguishing along the PC2 was trichome density in abaxial. This result indicates that the variable of trichome density could be used for separating the population of the species studies.

The scatter plot of the first two components from the principal component analysis can separate the population of *E. longifolia* based on trichome morphology and density (Figure 5). The first component was clustered Sentajo and Tahura population into the first group, and rest populations were clustered into second groups (axis x). The second component was separated the West Borneo, Lingga-1, and Sentajo populations with Tahura, Lingga-2, and Pokomo populations (axis y). This scatters plot of

PC1 and PC2 considered acceptable for discriminating of the *E. longifolia* population due to explained 99.78% of the total variation. The similar result was reported in *Eurycoma apiculata*, A.W Benn (PC1-PC2= 90%) [35]; and *Eurycoma longifolia* Jack (PC1-PC2= 80.16%) [3].

Table 5. Principal component analysis for trichome character of *E. longifolia*.

| Position | Variables | PC1 | PC2 | PC3 | PC4 |
|----------|---------------------|-------------|-------------|-------|--------|
| Abaxial | Peltate | 0.00 | 0.01 | 0.03 | -0.05 |
| | Capitate - Type I | 0.00 | 0.01 | 0.11 | 0.07 |
| | Capitate - Type II | 0.01 | 0.03 | -0.11 | -0.08 |
| | Capitate - Type III | 0.00 | -0.01 | -0.02 | 0.07 |
| | Trichome Density | 0.13 | 0.98 | 0.15 | 0.05 |
| Adaxial | Peltate | 0.00 | 0.00 | -0.01 | -0.01 |
| | Capitate - Type I | -0.02 | -0.05 | 0.03 | 0.91 |
| | Capitate - Type II | 0.06 | 0.11 | -0.72 | -0.23 |
| | Capitate -Type III | 0.00 | -0.09 | 0.66 | -0.32 |
| | Trichome Density | 0.99 | -0.13 | 0.02 | 0.03 |
| | Eigenvalue | 5095.47 | 491.90 | 8.12 | 3.39 |
| | Proportion | 91.01 | 8.79 | 0.15 | 0.06 |
| | Cumulative (%) | 91.01 | 99.79 | 99.94 | 100.00 |

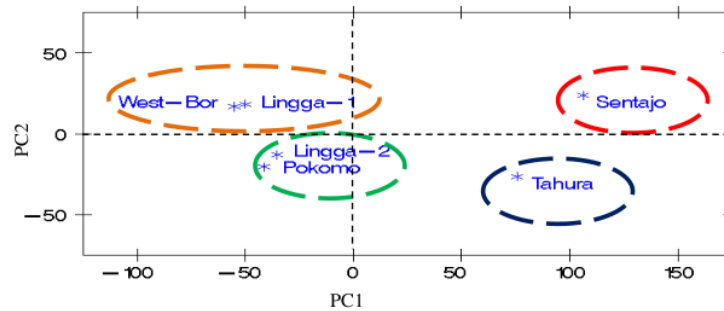


Figure 5. Scatter plot of PCA of *E. longifolia* populations.

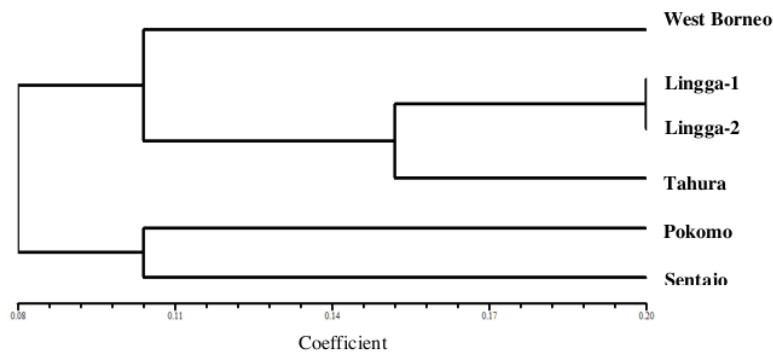


Figure 6. UPGMA dendrogram of *E. longifolia* based on similarity coefficient of trichome character.

The UPGMA dendrogram of *E. longifolia* based on the similarity coefficient of micromorphology characters among populations is exhibited in Figure 6. The UPGMA dendrogram of *E. longifolia* slightly different from the scatter plot of PCA projection. At the coefficient similarity among populations was 0.14, the UPGMA dendrogram of *E. longifolia* divided populations studied into four groups, the first and second groups were Sentajo and Pokomo populations, respectively, the third groups consisted of Lingga-1, Lingga-2, and Tahura populations, and the fourth groups was West Borneo population.

4. Conclusion

Two types of glandular trichomes were detected in leaflet *E. longifolia*, namely peltate and capitate trichomes. Trichome density in the adaxial leaflet surface is higher than the abaxial leaf surface and varies between populations. Trichome density in the abaxial was a positive correlation with mean annual precipitation whereas trichome density in the adaxial was a positive correlation with mean annual temperature. The variable of Trichome density can be used for separating the population of the species studies.

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