

Genetic Analysis of Itchy Leaves (*Laportea*, sp) in Papua for Herbal Medicinal Products as Development of Studying Economics Value

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Abstract

Papua is rich in biodiversity. The indigenous Papuans utilize most of endemic plants as food, board and medicines. One of the plants used as medicine is *Laportea*, sp, that locally known as *Daun Gatal*. The plant however, has not been developed commercially; this might due to lack of scientific providence of medicine content in this plant. The aims of this research is to (1) explore the types of *Laportea* growing in Papua, (2) conduct bio molecular character, which observes phylogeny relationship or genetic morphology in order to obtain data on the number of species found in Manokwari and Nabire. Bio molecular analysis is performed by PCR. Genetic analysis in this study was conducted through several phases, as follow: (a) Isolation of genomic total, (b) amplification of genes that encoded Rbcl, (c) sequencing the nucleotide sequence of the gene Rbcl of *Laportea*, (d) phylogeny analysis. The results showed that the DNA quality was good enough to do the next analysis. Of the four samples, two obtained haplo types are Haplo Type 1 (DT.NBX.H, DT.NBX.U and DR.MKW.H) and Haplo Type 2 (DR.NBX.L). The results of the analysis of phylogeny trees showed samples at 1 in the same haplo type, clade sequences of the genus *Dendrocnode* spp, *Discocnode*, and *Laportea*, while the sample at 2 in the same haplotype clade with *Laportea interrupta*, *Laportea ruderalis* and *Urera* sp.

Keywords

Genetics, Medicinal Herbs, Daungatal (*Laportea*, sp)

1. Introduction

Indonesia is a country that is extremely rich in biodiversity of medicinal plants

and it is potential to be developed. There are about 30.000 species of plants of the world's total of 40.000 plant species and 940 types are medicinal plants. This amount represents 90% of the medicinal plants in Asia. Many types of new drugs 20% - 22% plants were cultivated, while around 78% was obtained through direct retrieval (exploration) of forest [1].

Papua is part of east Indonesia. It obtains a huge number of biological state, which is about 20.000 - 25.000 species of plants and 142 species of them are endemic Papua [2]. The indigenous people of Papua utilize these plants as food, board and medicines. One of the plants used as medicine is *Laportea*, sp, the Papan people called *daun gatal* and scientifically known as *Laportea*, sp.

The results showed that *laportea* Decumana (Roxb) Wed was positive for alkaloids, glycosides, steroids/triterpenoids and negative for saponis, flavonoids and tannins [3]. Then formic acid was influencing the regardless pore dilation thus stimulating blood circulation to relieve aches. In addition to the tribe, some of *Laportea* are also used to relieve nausea or dizziness pat on the head, and relief pain during child birth.

This plant is utilized by nearly all the tribes in Papua. This plant grows in the highlands and lowlands. Utilization is only through direct exploration of nature without a process of cultivation. Based on research of population under the plant, there are three types of *laportea*, but until now there has been no study of bio molecular about all three types of plants, while according to Esiett, *et al.* [4], the Family of *Urticaceae* consists of 22 species. Therefore, it is necessary to conduct a study to analyze the molecular of *Laportea* in Papua. This study aims to (1) perform the ekspolrasi types of *Laportea* that grows in Papua, (2) do character the biomolecular, (3) obtain teaching materials for lectures stratum-1 and (4) disseminate the results of research in the national journal accredited or international Journal.

Laporeta have wide deployment; this plant grows in almost all regions. But until now, there has not been known how many species are found in Papua and which type has the highest content. Therefore, knowing the type and content of the highest is the target of this research.

Laportea used by Papuans as pharmaceuticals but until now there has been much research done on this plant and is not engaged in a professional manner. *Laportea* Commercialized in the market at a price of 5000 rupiah for 5 - 10 pieces. Commercialized parts of the plant are the leaves of of *Laportea*. *Laportea* leaves are commercialized taken directly from nature without cultivation that need to be developed with input cultivation of this plant fertilization can increase the content of useful is the target of this research. Harvesting of medicinal plants directly from natural habitat has threatened the conservation of several species of medicinal plants [5]. There are 55 species of medicinal plants are scarce in Indonesia with the status of scarcity [6].

Currently, the needs of herbal medicines is increasing, this is due to the lack of side effects from the use of herbal remedies based on Ditjen PEN, 2014. Industrial development of medicinal plant raw material in the last 5 years has shown

significant growth and earnings of production during this period increased by 2.5% - 30%/year. Therefore, the lack of scientific information about this plant it is important to conduct research on plants of *Laportea* assessment form that plants in Papua.

2. Materials and Methods

2.1. Sample Collection

The leaves were collected from Manokwari (West Papua Province) and Nabire (Papua Province). Fresh leaves were preserved and dried quickly by using silica gel.

2.2. Genomic DNA Isolation

Total genome of the sample leaf was isolated with *Genomic DNA mini kit (plant)*, Geneaid, by using standard protocol of the product. Shortly, the DNA isolation was done by taking approximately 50 - 100 mg of sample and grinding with sterile mortar. The grinded sample was added to the lysis buffer GPX1 (400 µL). To degrade the RNA, RNA-se was added to the solution and incubated at 65°C for five minutes. Before filtration, 100 µL of GP2 buffer was added then leaved on ice for three minutes. Filtration on filter column with centrifuge at 3500 rpm for 1 minutes. Supernatant of the filtrate was transferred to new micro centrifuge tube 1.5 mL. Then, buffer GP3 (1.5 times volume of supernatant) was mixed with the solution by vortex. The mixture was transferred to the GD column and centrifuge at 15,000 rpm for two minute. The GD column was washed with W1 buffer (400 µL) and wash buffer (600 µL) and each step was centrifuged at 15,000 rpm for 30 seconds. To dry the column, it was centrifuge at 15,000 rpm for three minutes. DNA genome that attached to the column was eluted with heated elution buffer (100 µL). The column and elution buffer were incubated at room temperature for three minutes to let the elution buffer interact with the DNA, then centrifuged at 15,000 rpm for 30 seconds. The extract DNA was kept in freezer.

2.3. Amplification of Ribulose 1,5 Difosfat Carboxylase Large Subunit Gene (*rbcL*)

Amplification of *rbcL* gen from *gatal* leaf was done with polymerase chain reaction (PCR) by using thermal cycler machine *MJ mini thermocycler*, model TN 1148, Biorad. The primer used were *rbcLaF* (5'-ATGTCACCACAAACAGAGACTA-AAGC-3') (Levin *et al.* 2003) and *rbcLaR* (5'-GTAAAATC AAGTCCACCRC-G-3') (Kres *et al.* 2009). The PCR reagents were Go-Taq-Green Master mix kit, Promega. PCR reaction was conducted in total volume 50 µL, that consist of 18 µL ddH₂O, 25 µL Go-Taq-Green master mix, 2.5 µL of primer *rbcLaF*, 2.5 µL of primer *rbcLaR*, and 1 µL of DNA genomic extract. The temperature profile of the PCR machine was 80°C for 10 seconds, 94°C for 2 minutes, then followed by 35 cycle of 3 steps which were (94°C for 30 seconds, 50°C for 30 seconds and 72°C for 1 minute), the final extension at 72°C for 5 minute, then closed with 37°C for one minute.

2.4. Electrophoresis

The visualization of PCR product was done by electrophoresis gel agarose 1% with sodium boric buffer and voltage 100 V for 30 minutes. Each well consist of 4 μ L PCR product and 1 μ L of loading dye. As the marker, Generuller 1 Kb DNA ladder (Thermoscientific) was used. The dying of DNA was conducted by immersing the gel on ethidium bromide solution for 20 minutes and visualize with UV-transilluminator. The gel was documented by digital camera.

2.5. Sequencing

The sequencing of *rbcl* genes is conducted by dideoxy termination sanger. The process was done by company service, PT. Genetika Science Indonesia, Jakarta. The electropherogram in ABI format sent by email.

2.6. Data Analysis

The sequence of *rbcl* gene was proof read with the electropherogram with MEGA 5 software [7]. Basic local search alignment tools (BLAST) from NCBI was used to compare our data with the GenBank data (NCBI). Phylogenetic tree analysis (*neighbor joining* and *maximum likelihood*) was made with MEGA 5 software. The bootstrap support in each branch was calculated by bootstrap methods with 1000 replication.

3. Results and Discussion

3.1. The Spread Pattern of *Laportea Spin Nabire* and Manokwari Regency

Based on the results of research conducted in the regency of Manokwari and Nabire result that *daungatal* or by the Latin name is *Laportea* sp, there is wide-spread in lowland up to the highland, the deployment begins at a height of 50 meters above sea level to 1500 meters above sea in the Manokwari regency. *Laportea* plants live in secondary forest and primary forest. In the primary forest, these plants live under stands of large trees with wet environmental conditions but dry land and have plenty of humus. This is consistent with the statement of the WHO [8] that this *Laportea* plant habitats in the shade and grows well in wet areas but with the dry land. In addition, this plant has been cultivated by some local communities in Manokwari in the yard of the house, where in addition to the needs of family medicine this plant is also traded in the market for profit. Similarly, in the Nabire regency, based on the exploration results obtained plants *Laportea* were living in primary forests and secondary forests that spread from the lowlands to the highlands of the area Wanggar-Lagari until the Topo-Dogiyai, which ranges at an altitude of 65 masl to 1300 masl. This plant in Nabire is not cultivated by the community because this plant grows wild and naturally so that the beneficiaries are taken directly from nature.

From the results of exploration in Manokwari and Nabire obtained some kind of *Laportea*. Based on altitude, in the district of Manokwari in the lowlands, three plant species of *Laportea* are found by the local name Consist thin *daungatal*, red

and the *Laportea pig.* While in the highlands also found that living plants of the *Laportea* that leaves itchy scratchy thin and red leaves. Based on the exploration results in Nabire, in the lowlands found three types of *laportea* living is based on local names *gatel* leaves, leaf green and *Laportea* scratchy purple, while living on the plateau that leaves itchy scratchy green and purple leaves. Leaf morphology itchy red and green leaves and flowers are shown in **Figure 1**.

3.2. Molecular Genetic Analysis

3.2.1. Genomic DNA of *Laportea*, Isp

A total of six samples of *Laportea* from Nabire and Manokwari (**Table 1**), has isolated its genome DNA by using genomic geneaid isolation kit (plant). The success of the isolation of genomic DNA is shown with their band on a 1% agarose gel electrophoresis. Sightings DNA bands of the six samples are presented in **Figure 2**.

Based on the results of electrophoresis in **Figure 2**, seen all the DNA samples had white ribbons lengthwise. The quality of the sample genome DGR.MKW.H are very good with a very thick color, which indicates the concentration of DNA obtained is very high. Other samples had sufficient quality to be used in the PCR process.

3.2.2. Amplicon Gene Ribulose-1,5-Bisphosphate-Carboxylase (Rbcl)

Gen Rbcl of *Laportea* in the leaf samples in vitro amplification using polymerase chain reaction (PCR). Primary used is Rbcl-Ar [9] and Rbcl-Af [10]. Electrophoresis was performed using 1% agarose gel. As the marker (marker) used DNA marker 1 kb. Long amplicons obtained had a size of about 400 bp. With DNA quality was very good with high levels of brightness, brighter in the DNA bands in DNA marker which has a concentration of 70 ng/uL for most bright ribbon of DNA markers. This means that the DNA amplicon concentration higher than 70 ng/uL. This concentration is enough to be used in the sequencing stage. The results of electrophoresis of products PCR can be seen in **Figure 3**.

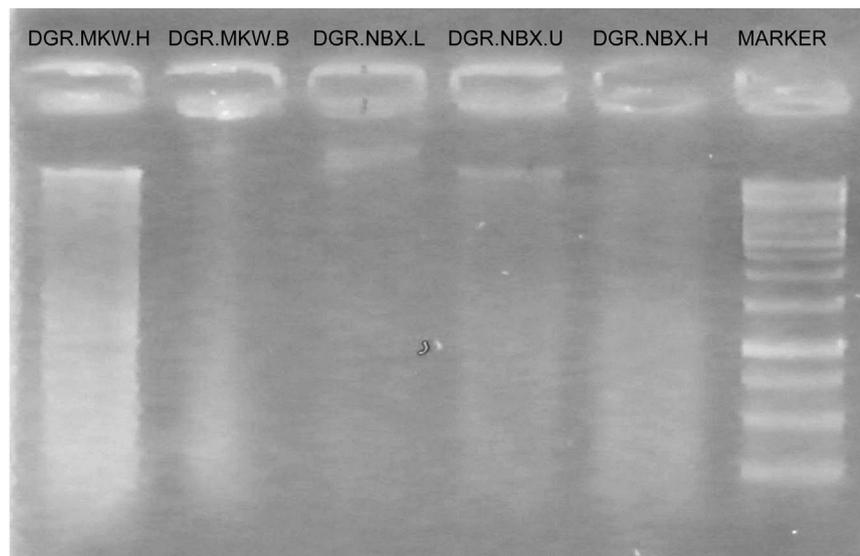
Electrophoresis results showed that there were four samples were successfully



Figure 1. Morphological of Plant *Laportea* sp living in primary forests *Okabai*, Topo district of Nabire Regency.

Table 1. List of samples used in molecular genetic analysis.

ID Sampel	Jenis	Lokasi
DGT.NBX.H	Daun gatal hijau	Dataran Tinggi Nabire
DGT.NBX.U	Daun gatal ungu	Dataran Tinggi Nabire
DGR.NBX.L	Daun gatal lateng	Dataran Tinggi Nabire
DGR.MKW.B	Daun gatal babi	Dataran Rendah Manokwari
DGR.MKW.H	Daun gatal hijau	Dataran Rendah Manokwari

**Figure 2.** Ribbon Genome DNA from leaves itchy origin Nabire and Manokwari.

amplified by using primer Rbcl-Ar and Rbcl-Af, are sample DT.NBX.H, DT.NBX.U, DR.NBX.L and DR.MKW.H, meanwhile, samples DR.MKW.B not succeed in amplification (results not shown). The length amplicon obtained about 800 base pairs. It is seen by aligning the position of the sample DNA with DNA markers of known length.

3.3. Phylogenetic Analysis

Sequencing of the four samples of *Laportea*, is derived nucleotide sequences that encode genes Rbcl. The sequence is checked and adjusted (proof reading) with elektroferogram obtained, to ensure the correct sequence. The process of proof reading is based on the forward and reverse sequences of each sample. The quality of the sequences obtained was excellent, as indicated by the high peaks and clean from elektroferogram.

The results alignment and the primary cutting edge, obtained along the 552 nucleotide base pairs. Of the four samples of *Laportea*, 17 points polymorphism obtained. The point of polymorphism is a nucleotide sequence that differs from one sequence to another. Based on the polymorphism, four samples can be classified into two haplotypes, a DNA sequence is said to be different haplotype when

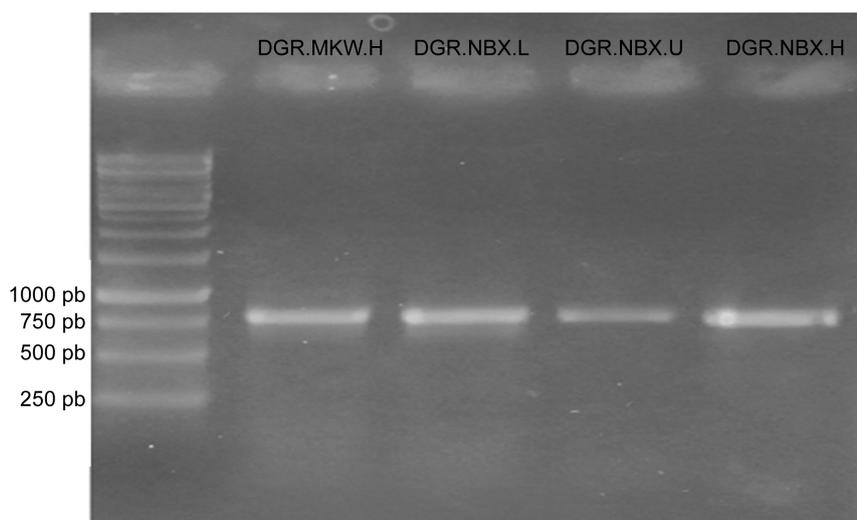


Figure 3. Results of electrophoresis of PCR products from leaf itch gene Rbcl origin Nabire and Manokwari.

there are one or more nucleotides different when compared with other sequences. The first consists of a sample haplotype DT_NBX_H, DT_NBX_U and DR_MKW_H, while the second is a sample DR_NBX_L haplotype. There were no nucleotide variations between samples of genes Rbcl green *Laportea* origin of Nabire with leaves of Manokwari, like wise for *Laportea* leaf green and purple. Nucleotide variation is found (in the genes Rbcl) between the leaves with *Laportea* lateng others.

Nucleotide variation is found (in the genes Rbcl) between the itch lateng leaves and the others of itch leaf. The variations are the result of a point mutation in the form of transition (Ts) and Transversions (Tv) (**Table 2**). Transition mutations are DNA mutations that occur due to changes in nucleotides of purine bases (adenine (A), guanine (G)) be a purine or pyrimidine bases (Cytosin (C), Thymine (T)) be a pyrimidine. While transversion mutation occurs due to a mutation of purine bases (adenine (A), guanine (G)) into pyrimidine (Cytosin (C), Thymine (T)) or reverse. Details of the mutated nucleotide position transitions and tranversi presented in **Table 2**.

Analysis of the phylo genetic tree created using MEGA software 5 [7]. Based on morphological analysis, obtained the basic information that *Laportea* samples in this study belong to the genus of *Laportea*. Therefore, as a reference in the manufacture of the phylogenetic tree, use some sequences of genes Rbcl from NCBI. According to Wu *et al.* [11], urticaceae family members can be grouped into three major clades. One clade of *Laportea* genus in which there is a third clade, in which there are genus *Nanocnide*, *Urtica*, *Girardinia*, *Dendrocnicide*, *Discocnicide*, *Poikilospermum*, *Urera*, *Touchardia*, *Obetia* and *Laportea*. Therefore, the phylo genetic tree made using gene Rbcl of genus-the genus are available at NCBI. A total of 420 base pairs of nucleotides that encode genes Rbcl of 89 sequences (4 samples of leaves itchy, one sequence out group, and 83 sequences from the family *Urticaceae* selected from NCBI). The phylo genetic tree was made by the method of maxi-

Table 2. Polymorphisms point of the fourth samples of *Daun gatal*.

ID Sample	Nama of Sample.	Nucleotida position								
		75	114	150	263	327	366	393	396	400
DT_NBX_H	D. Gatal Hijau	C	A	T	T	T	C	A	C	T
DT_NBX_U	D. Gatal Ungu	C	A	T	T	T	C	A	C	T
DR_NBX_L	D. Gatal Lateng	T	C	A	A	G	G	C	T	G
DR_MKW_H	D. Gatal Hijau	C	A	T	T	T	C	A	C	T
	Mutation	Ts	Tv	Tv	Tv	Tv	Tv	TV	Ts	Tv

ID Sample	Name of Sample	Nucleotida position							
		406	407	408	417	435	471	522	537
DT_NBX_H	D. Gatal Hijau	A	C	T	C	T	C	G	A
DT_NBX_U	D. Gatal Ungu	A	C	T	C	T	C	G	A
DR_NBX_L	D. Gatal Lateng	G	T	C	T	C	T	A	T
DR_MKW_H	D. Gatal Hijau	A	C	T	C	T	C	G	A
	Mutation	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Tv

imum likelihood parameter K2P + G (Kimura 2 parameter and Gamma distribution). The meter is chosen from the test results the appropriate evolutionary model by using model test [10] in the MEGA 5. Bayesian Inference model with the smallest value is selected as the best model [12]. Bootstrap 1000 replication method used to test the phylo genetic trees were created. Making the results presented in the phylo genetic tree in **Figure 4**, value of each branch boot strapt written on each base branching. This value indicates the support or the confidence level of each branch formed 1000 repetitions building the tree.

Phylogenetic tree in **Figure 4** indicates that three samples of leaves itchy with ID DT.NBX.H, DT.NBX.U and DR.MKW.H are in the same clade. This indicates that based on data from three samples Rbcl gene is one species to the same. Although the morphology of bone samples DT.NBX.U has leaves and the underside of leaves are purple, but the nucleotide sequence of its gene Rbcl exactly, in addition, in the clade seen the clade of the genus group of *Dendrocnidaespp*, *Laportea* and *Descocnidemexicana*. Value the support of the establishment of branches between the sample and sequences spp *Dendrocnidae* very small at only 31%. With the presence of several species are joined into one large clade and formed politomi, namely branching comb, accompanied by a low boot strapt indicates that the kinship of the three groups did not seen any real difference. This means that, Rbcl gene which is a DNA barcoding for plants could not distinguish between the third genus.

Samples DR.NBX.L, in phylogenetic, are in a clade with *Laportea interupta*, *L. ruderalis* and *Urera* sp. with bootstrap 66%. The third ramification of this species

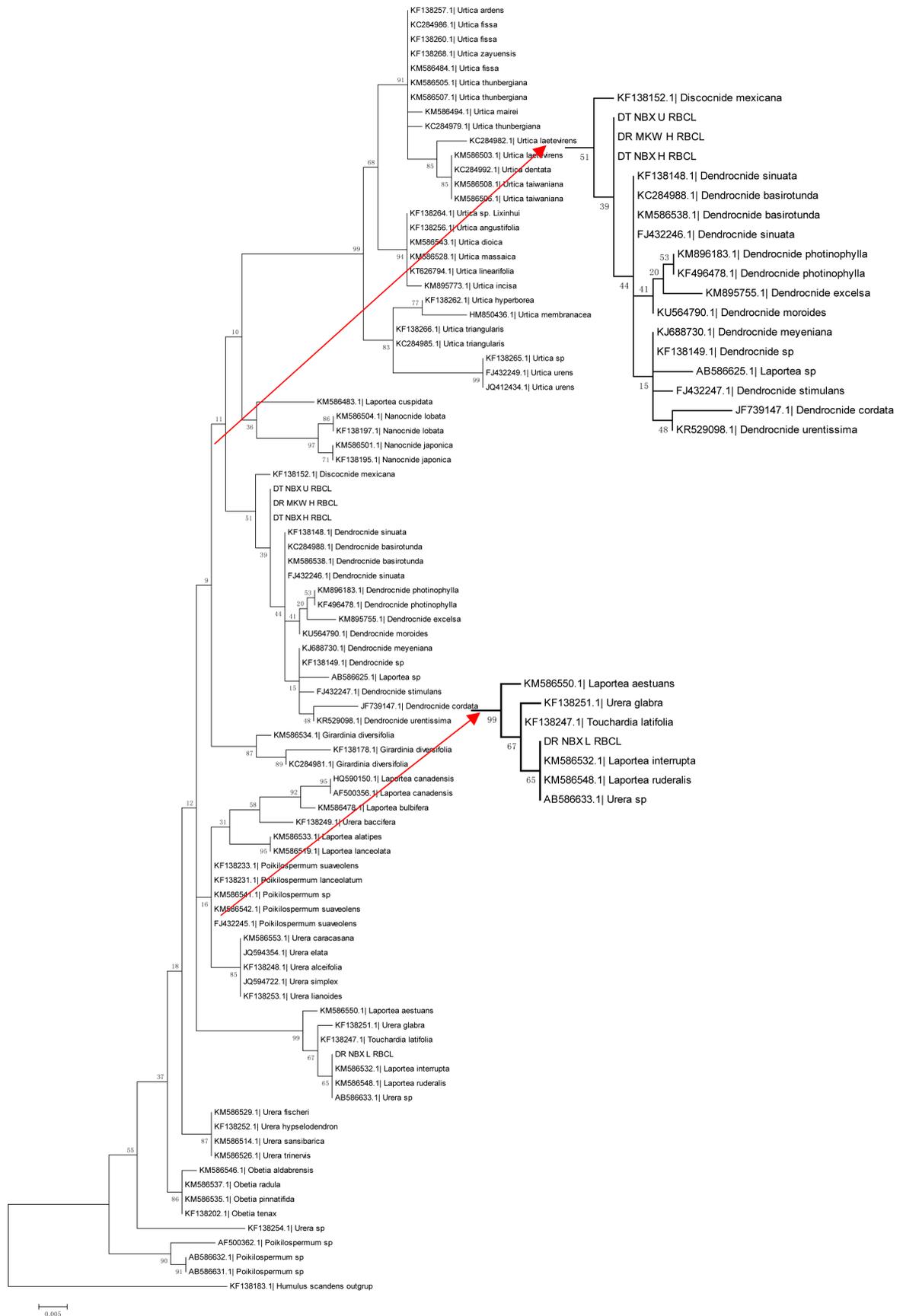


Figure 4. Phylogenetic tree.

also are politomi, so the kinship of all three cannot be distinguished by using gene Rbcl. Based on morphological analysis, it appears that this sample is a sample of the species *Laportea interrupta*. With the results of phylogenetic tree analysis shows that this sample are in a clade with *L. interrupta*, so it can be sure that the species is *L. interrupta*, although in the clade there are other species. Therefore, it is necessary to do further research that examines the kinship and systematics of the group leaves from Papua itchy by using some other loci, both chloroplast and nuclear DNA locus.

4. Conclusions

1) The nucleotide sequence of the gene Rbcl *Laportea* origin Nabire and Manokwari successfully determined the sequence with a total length reaching 552 base pairs.

2) Of the four samples, two obtained haplotype are Haplo Type 1 (DT.NBX.H, DT.NBX.U, DR.MKW.H) and Haplo Type 2 (DR.NBX.L).

3) The results of the phylogenetic tree analysis show samples at 1 in the same haplo type and clade sequences of the genus *Dendrocnides* spp, *Discocnide*, and *Laportea*, while the sample at 2 in the same haplotype clade with *Laportea interrupta*, *Laportea ruderalis* and *Urera* sp.

4) Gen Rbcl of the results of this study cannot explain the systematic relationships of these samples to the extent of the species. Multigene analyses or multilocus necessary are able to explain the systematics of the group leaves itchy.

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