




## GENOME NOTE

# The complete chloroplast genome of a centennial olive tree (*Olea europaea*, Oleaceae) from the southern Peruvian coast

[version 1; peer review: 1 approved, 1 approved with reservations]

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## Abstract



Olive (*Olea europaea* Linnaeus 1753) is one of the valuable fruit trees and very important edible oil plant in the world. The chloroplast (cp) genome of an olive tree (*Olea europaea*) from the southern Peruvian arid coast was obtained for the first time. Genomic DNA of high quality was used to generate libraries with Illumina HiSeq paired-end methods. The cp genome is 155,886 pb in length and contains a large single-copy region (LSC) of 86,610 pb and a small single copy region (SSC) of 17,790 pb separated by two inverted repeat (IR) regions (25,741 bp). The cp genome of olive contains 124 genes that consists of 80 protein-coding genes, 36 tRNA, eight rRNA. Phylogenetic analysis showed this olive tree is sister to *O. europaea* subsp. *maroccana* (*Oleae* tribe). This study presents the first overview of the chloroplast genome organization and phylogenetics of *O. europaea*, offering valuable insights for genetic and evolutionary research in the genus *Olea*.


## Keywords


plastome, NGS, coastal zone, phylogenomics

## Open Peer Review

Approval Status  

	1	2
<b>version 1</b>		
04 Dec 2024	<a href="#">view</a>	<a href="#">view</a>

1. **Jun Kim** , Chungnam National University, Daejeon, South Korea

2. **Rajagopal Kammara** , Central Food Technological Research Institute, Mysore, India

Any reports and responses or comments on the article can be found at the end of the article.

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**Author roles:** **Saldaña CL:** Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Chura-Llanos LS:** Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Estrada R:** Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Fernandez E:** Investigation, Writing – Review & Editing; **Zirena Vilca F:** Investigation, Resources, Writing – Review & Editing; **Guerrero-Abad JC:** Investigation, Methodology, Writing – Review & Editing; **Amasifuen-Guerra CA:** Investigation, Writing – Review & Editing; **Gutierrez-Reynoso DL:** Investigation, Writing – Review & Editing; **Injante P:** Investigation, Writing – Review & Editing; **Arbizu CI:** Investigation, Methodology, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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## Introduction

The olive *Olea europaea* (Oleaceae) is a subtropical tree distributed on most continents in tropical and temperate environments (Dupin et al., 2020). The genus *Olea* comprises about 40 old world species (Jensen et al., 2002) and the majority of Oleaceae species possess an economic importance as most olive fruits are used for oil extraction. Vegetable oil is important for its nutritional and healthy advantages compared to others (Rallo et al., 2000). Currently, ten olive tree chloroplast (cp) genomes were reported from Mediterranean region (Besnard et al., 2011), Italy, Spain, China (Niu et al., 2020), and Jordan (Haddad et al., 2021). However, to date, the olive tree chloroplast genome from Peru has not been reported. In this study, we sequenced, assembled, and annotated for the first time the complete chloroplast genome of a centennial the olive tree from Peru (Figure 1), using next generation sequencing (NGS), providing valuable information for genetic and evolutionary studies in the genus *Olea*.

## Methods

**Plant materials and extraction of genomic DNA** Fresh young leaves were collected from “El Algarrobal” district located in the Ilo province from Moquegua region (latitude 17.61294, longitude -71.27136). The specimen (MOQ001169) was deposited at the National University of Moquegua Herbarium (<http://www.hmoqueguensis.unam.edu.pe/>, Hibert Huaylla-Limachi [hmoqueguensis@unam.edu.pe](mailto:hmoqueguensis@unam.edu.pe)) under the voucher Nro. 001169. DNA was extracted using CTAB method (Doyle and Doyle 1990) with minor modifications for this specie. The quality and the quantity were evaluated on a 1% agarose gel and fluorescence using the Qubit™ 4 Fluorometer (Invitrogen, Waltham, MA, USA), respectively.

**DNA sequence and genome assembly** Library construction pair-end reads and sequencing were carried out using Illumina HiSeq 2500 platform and a PE 150 library, with the NexteraXT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). We removed the adapters and verified the quality of reads employing Trim Galore (Martin, 2011) with arguments: `-F embplant_pt -R 15 -reduce-reads-for-coverage inf`. The cp genome was assembled with GetOrganelle v1.7.2 (Jin et al., 2020), using *Olea europaea* subsp. *europaea* (NC\_015401) as reference. SPAdes v3.11.1 (Bankevich et al., 2012), bowtie2 v2.4.2 (Langmead and Salzberg, 2012), and BLAST+ v2.11 (Camacho et al., 2009) were also used in the pipeline with default settings.

## Annotation of *Olea europaea* CP genome

The cp genome was annotated using GeSeq in CHLOROBOX web service (Tillich et al., 2017) Default settings were applied, and comparisons were made with all available plastid genomes of Oleae in the NCBI database, followed by manual curation

## Phylogenetic analyses

To understand the phylogenetic position of *Olea europaea*, a maximum likelihood (ML) tree of 19 genomes retrieved from GenBank was reconstructed. First, we employed MAFFT v.7.475 (Katoh and Standley, 2013) to align those genomes. Then, with a GTR + GAMMA model of evolution, we obtained the best-scoring ML tree, considering 1,000 bootstrap (BS) inferences with RAxML v8.2.11 (Stamatakis, 2014). We employed capirona (*Calycophyllum spruceanum*) cp genome as an outgroup (OK326865). The aligned data that was employed for the phylogenetic analysis consists of 187,111 bp (Supplemental Data 1, <https://doi.org/10.5061/dryad.tmpg4f57q>).

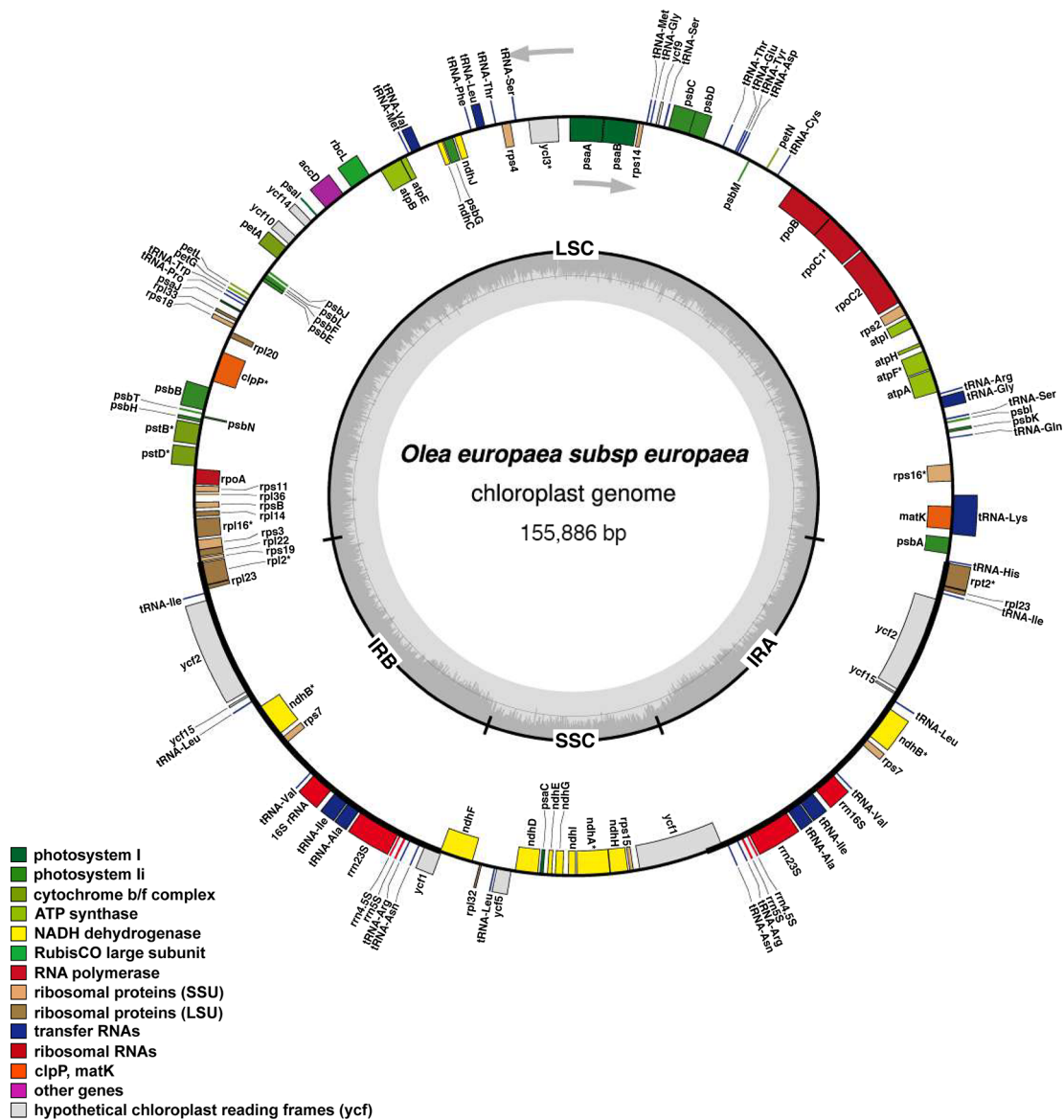


**Figure 1.** Picture of *Olea europaea*, a centennial olive tree from Moquegua, Peru.

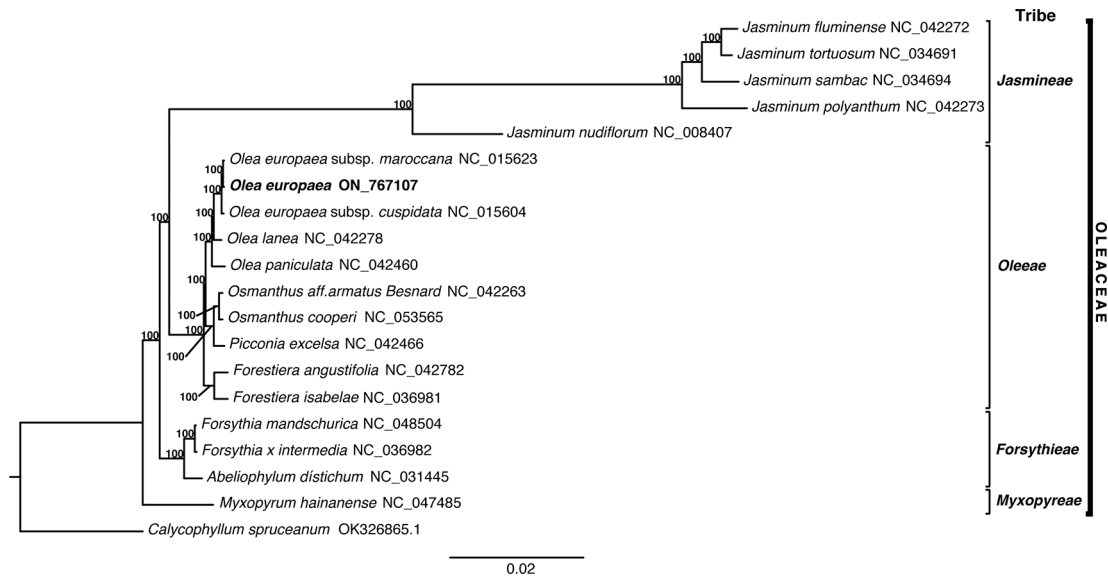
## Results

### *Olea europaea* chloroplast genome assembly

The complete chloroplast genome of olive was 155,886 bp in length with typical quadripartite structure that included a large single-copy (LSC) of 86,610 bp and a small single copy region (SSC) of 17,790 bp separated by two inverted repeat (IR) regions (25,741 bp). Following of annotation and subsequent modifications, we submitted the complete chloroplast (cp) genome sequence to the GenBank database. This submission is associated with the accession number: ON767107. The average depth of coverage is 17,673.05X (Supplementary Figure 1, <https://doi.org/10.5281/zenodo.14061216>). The chloroplast genome contains 124 genes that consisted of 80 protein-coding genes, 36 tRNA, 8 rRNA. Of the total genes reported 12 present one intron. *PafI* and *clpP1* genes contained two introns. We further report that most genes are present as a single copy, except 14 genes that were duplicated in IR regions (Figure 2). A total of 11 cis-splicing genes (*rps16*, *atpF*, *rpoC1*, *pafI*, *clpP1*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, *ndhA*) were identified; one trans-splicing gene (*rps12*) was also identified (Supplementary Figure 2, Supplementary Figure 3).



**Figure 2. Schematic map of features of *Olea europaea* chloroplast genome (Genes drawn outside the outer circle are transcribed clockwise, and those inside are transcribed counter-clockwise. Genes belonging to different functional groups are color-coded. The genes functions are indicated colored in the bottom left corner. The dark grey inner circle indicates the presence of nodes in the LSC, SSC, IR regions).**



**Figure 3.** The phylogenetic position for *O. europaea* according to the ML phylogenetic tree constructed based on 19 chloroplast genomes: *Jasminum tortuosum* NC\_034691 (Unpublished), *Jasminum sambac* NC\_034694 (Unpublished), *Jasminum fluminense* NC\_042272 (Olofsson et al., 2019), *Jasminum nudiflorum* NC\_008407 (Lee et al., 2007), *Jasminum polyanthum* NC\_042273 (Olofsson et al., 2019), *Olea europaea* subsp. *maroccana* NC\_015623 (Besnard et al., 2011), *Olea europaea* subsp. *cuspidata* NC\_015604 (Besnard et al., 2011), *Olea lancea* NC\_042278 (Olofsson et al., 2019), *Olea paniculata* NC\_042460 (Olofsson et al., 2019), *Osmanthus aff. armatus* Besnard NC\_042263 (Olofsson et al., 2019), *Osmanthus cooperi* NC\_053565 (Wang et al., 2019), *Picconia excelsa* NC\_042466 (Olofsson et al., 2019), *Forestiera angustifolia* NC\_042782 (Olofsson et al., 2019), *Forestiera isabelae* NC\_036981 (Van de Paer et al., 2018), *Forsythia mandschurica* NC\_048504 (Olofsson et al., 2019), *Forsythia x intermedia* NC\_036982 (Park et al., 2019), *Abeliophyllum distichum* NC\_031445 (Kim et al., 2016), *Myxopyrum hainanense* NC\_047485 (Zhu et al., 2020), *Calycophyllum spruceanum* OK326865 (Saldaña et al., 2022).

### Phylogenetic inference of *Olea europaea*

This study examined 19 species of the Oleaceae family, with one outgroup, *Calycophyllum spruceanum* (OK326865), to understand their evolutionary relationships by analyzing complete chloroplast genome sequences. Maximum likelihood (ML) analysis revealed three distinct monophyletic groups within the tribes Jasmineae, Oleae, and Forsythieae, with 17 of the nodes showing 100% bootstrap support. In addition, *O. europaea* is shown to be sister to *O. europaea* subsp. *maroccana*, and sister to them is *O. europaea* subsp. *cuspidata* (Figure 3). As expected, *Olea europaea* was categorized in the Oleae tribe. These phylogenetic trees are consistent with the established classification of the Oleaceae family.

### Discussion and conclusions

In this study, we assembled for the first time the chloroplast genome sequence of the centennial olive tree (*Olea europaea*) from the southern Peruvian coast. The results differ from other chloroplast genomes of *Olea europaea*, such as *O. europaea* subsp. *europaea* (NC\_015401), which presents 85 protein-coding genes, 37 tRNA, and 8 rRNA (Haddad et al., 2021). Niu et al. (2020) reported that two varieties of *O. europaea*, subsp. *europaea* var. *sylvestris* and subsp. *cuspidata*, presented 133 genes, including 87 protein-coding genes, 37 tRNA, and 9 rRNA. Likewise, they found that the chloroplast genome length for both species were 155,886 bp (Niu et al., 2020). The phylogenetic results showed *O. europaea* is sister to *O. europaea* subsp. *maroccana* and *O. europaea* subsp. *cuspidata* in independent sister clade to other species of genus *Olea* the chloroplast genome sequence and annotation were submitted to NCBI with accession number ON767107.1.

Peruvian olive chloroplast genome will stimulate additional work to develop molecular markers for their application in a genomic selection program of superior individuals in early stages. In addition, this study may be a basis to know the location of genes of interest that may be used in gene editing. This work promotes knowing more about the genetics of the Peruvian olive tree, promoting its modern genetic improvement and conservation.

### Authors' contribution

C.L.S. analyzed the data, was also involved in drafting the article. L.C.-L., C.I.A. and R.E. analyzed the data. E.F.-H., F.Z.-V., J.C.G.-A., C.A.-G., D.L.G.-R. were involved in the conception and design of the work. L.C.-L. and F.Z.-V. were involved in sample collection. F.Z.-V., J.C.G.-A., P.I. and C.I.A. were involved in data interpretation.

F.Z.-V., J.C.G.-A., P.I. and C.I.A. were involved in funding acquisition. C.I.A. was involved in drafting the article. All authors read and approved the manuscript.

### Ethical approval

Olive sample collected and employed in this work do not involve protection determined by the Republic of Peru. Consequently, this study was exempted from ethical approval.

### Data availability statement

#### Underlying data

NCBI: Complete chloroplast genome. Accession number ON767107; <https://www.ncbi.nlm.nih.gov/nuccore/ON767107>.

#### Extended data

Dryad: Supplementary material.

The complete chloroplast genome of a centennial olive tree (*Olea europaea*, Oleaceae) from the southern Peruvian coast. <https://doi.org/10.5061/dryad.tmpg4f57q> (Saldaña et al., 2024a).

This link contains the following extended data:

- Supplementary\_Figure\_1.jpg
- Supplementary\_Figure\_2.jpg
- Supplementary\_Figure\_3.jpg

Zenodo: Supplementary material. <https://doi.org/10.5281/zenodo.14061216> (Saldaña et al., 2024b).

This link contains the following extended data:

- Supplementary\_Data\_1.phy
- README.md

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication) (<http://creativecommons.org/publicdomain/zero/1.0/>).

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# Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 28 January 2025

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**Rajagopal Kammara** 

Central Food Technological Research Institute, Mysore, India

The major objective is understanding the genomics of *Olea europaea chloroplast genome*. It is novel, and not yet reported from Peru. Thorough discussion at genome level emphasizing the secondary metabolite pathways etc., is not discussed it requires more emphasis. It is well documented that the value of this tree is tremendous by showing uniqueness at genome level may bring more readership and very useful globally. Therefore, the above said needs to be discussed further.

### Modifications required

1. Genomic DNA of high quality was used to generate libraries with Illumina Hiseq paired-end methods
2. The cp genome of olive contains 124 genes that consists of 80 protein-coding genes, 36 tRNA, eight rRNA  
36 tRNA, eight rRNA ended as it is an incomplete sentence.
3. Following of annotation and subsequent modifications, we submitted the complete chloroplast (cp) genome sequence to the GenBank database.
4. Of the total genes reported 12 present one intron
5. Figure 2. Schematic map of features of *Olea europaea* chloroplast genome (Genes drawn outside the outer

Needs to fill the gaps.... **Incomplete Gaps need to be filled in the MS**

1. A briefing of the Genomic DNA extraction procedure is required as it is the backbone of the manuscript
2. The presence of Secondary metabolite pathways needs to be emphasized
3. The emphasis at the genome level to show that the Peruvian *Olea europaea* tree is unique

**Are the rationale for sequencing the genome and the species significance clearly described?**

Partly

**Are the protocols appropriate and is the work technically sound?**

Partly

**Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?**

Partly

**Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Genomics, Proteomics and bacteriocins, smart probiotics

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 23 January 2025

<https://doi.org/10.5256/f1000research.170279.r357668>

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**Jun Kim** 

Chungnam National University, Daejeon, South Korea

Thank you for the opportunity to review this manuscript, which reports the chloroplast genome of a centennial olive tree. It is commendable that the authors have submitted their data to the NCBI database, making it freely accessible to other researchers. With recent advancements in long-read sequencing technologies, it is now possible to assemble entire genomes, not just chloroplast genomes. I look forward to the potential publication of a complete genome assembly of this species in the future.

I have noted a few minor issues in the manuscript. For future submissions, I strongly recommend that the authors utilize a proofreading or editing platform. Specific points are as follows:

[Abstract]

Genomic DNA of high quality was used to generate librarieswith  
--> libraries with

a large single-copy region (LSC) of 86,610 pb and a small single copy region (SSC)  
--> single-copy region (SSC)

155,886 pb

--> bp  
17,790 pb  
--> bp

#### [Introduction]

In this study, we sequenced, assembled, and annotated for the first time the complete chloroplast genome of a centennial the olive tree from Peru ( Figure 1)  
--> (Figure 1)

#### [Methods]

Plant materials and extraction of genomic DNA Fresh young leaves were collected  
--> "Plant materials and extraction of genomic DNA" should be bold and should include a "\n" at the end to break up an awkwardly joined sentence.

with minor modifications for this specie  
--> species

DNA sequence and genome assembly Library construction  
--> "DNA sequence and genome assembly" should be bold and should include a "\n" at the end to break up an awkwardly joined sentence.

—F embplant\_pt —R 15 —reduce-reads-for-coverage inf  
--> The en dash ("—") might be "--"

The cp genome was annotated using GeSeq in CHLOROBOX web service (Tillich et al., 2017)  
Default settings were applied, and comparisons were made with all available plastid genomes of Oleae in the NCBI database, followed by manual curation  
--> These sentences need to end with a period (".").

#### [Results]

To understand the phylogenetic position of *Olea europaea*, a maximum likelihood (ML) tree of 19 genomes retrieved from GenBank was reconstructed.  
--> When referring to "19 genomes," please specify the taxon (likely the Oleaceae family).  
Additionally, the relevant accession numbers shown in Figure 3 should be also mentioned in this sentence.

The average depth of coverage is 17,673.05X  
--> Please replace "X" with the multiplication symbol "×" where appropriate.

In addition, *O. europaea* is shown to be sister to *O. europaea* subsp. *Maroccana*, and sister to them is *O. europaea* subsp. *cuspidata* ( Figure 3)  
--> (Figure 3)

**Are the rationale for sequencing the genome and the species significance clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Genome assembly using long-read sequencin

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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