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Morphological and molecular characterization of *Stenocranus pacificus* (Hemiptera: Delphacidae) on maize plants

Karakterisasi morfologi dan molekuler *Stenocranus pacificus* (Hemiptera: Delphacidae) pada tanaman jagung

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ABSTRACT

Maize (*Zea mays L.*) is one of the primary food commodities with a strategic role in improving Indonesia's economy. Additionally, maize contributes to food diversification and security. One of the main threats to maize production is pest attacks, including *Stenocranus pacificus* and *Peregrinus maidis*. This study aims to identify *S. pacificus* (Hemiptera: Delphacidae) found on maize crops in three sub-districts of Lamongan Regency, East Java, using both morphological and molecular approaches. Morphological identification revealed that the planthopper found is *S. pacificus*, characterized by an orange abdomen in male imagos and a white abdomen in female imagos. Molecular testing through polymerase chain reaction (PCR) using mitochondrial Cytochrome Oxidase I deoxyribonucleic acid (mtCOI DNA) showed that the *S. pacificus* from Lamongan is 99.53% similar to *S. pacificus WRJJ_UNILA* and *WRJB_UNILA*. These findings are expected to support more accurate, rapid, and effective pest control strategies.

ABSTRAK

Jagung (*Zea mays L.*) merupakan salah satu komoditas pangan utama yang memiliki peran strategis dalam meningkatkan perekonomian Indonesia. Selain itu, jagung juga berperan dalam diversifikasi pangan dan ketahanan pangan. Salah satu ancaman utama bagi produksi jagung adalah serangan hama, termasuk *Stenocranus pacificus* dan *Peregrinus maidis*. Penelitian ini bertujuan untuk mengidentifikasi hama wereng *S. pacificus* di lahan jagung di tiga kecamatan di Kabupaten Lamongan, Jawa Timur, Indonesia, melalui pendekatan morfologi dan molekuler. Identifikasi morfologi menunjukkan bahwa wereng yang ditemukan adalah *S. pacificus*, dengan imago jantan yang memiliki abdomen oranye dan imago betina yang memiliki abdomen putih. Pengujian molekuler menggunakan *polymerase chain reaction* (PCR) dengan *mitochondrial Cytochrome Oxidase I deoxyribonucleic acid* (mtCOI DNA) menunjukkan bahwa *S. pacificus* dari Lamongan memiliki tingkat kemiripan 99.53% dengan *S. pacificus WRJJ_UNILA* dan *WRJB_UNILA*. Hasil penelitian ini diharapkan dapat mendukung pengendalian hama yang lebih tepat, cepat, dan efektif.

INTRODUCTION

Maize (*Zea mays L.*) is one of the primary food commodities in Indonesia, playing a vital and strategic role in the country's economy (Ramayana et al., 2021). Maize contributes significantly to food diversification, enhances food security, and reduces dependence on other staple crops. In Indonesia, the demand for maize continues to rise. According to data from the Central Bureau of Statistics (BPS, 2023), the production of dry-shelled maize with 14% moisture content in 2023 reached 14.46 million tons, marking a decline of 1.25% compared to the 16.53 million tons produced in 2022.

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In the context of cultivation, maize is highly susceptible to attacks by plant pests and diseases, which can drastically reduce crop yields (Siallagan et al., 2021). These pests include various insects, diseases, and weeds that cause damage to crops (Virla et al., 2021). Among the most frequent pests attacking maize are the planthoppers, particularly *S. pacificus* and *Peregrinus maidis* (Neves et al., 2022). *S. pacificus* is considered a significant pest capable of causing substantial damage to maize crops in Indonesia. The damage caused by *S. pacificus* can result in "hopperburn," leading to crop failure. Symptoms include leaf chlorosis, necrosis, stunted growth, and weakened plant vitality (Hasibuan et al., 2021). This pest feeds on plant sap from the phloem, damaging the plant's tissues. Although *S. pacificus* has not been reported as a vector of plant pathogens, the physical damage it causes can significantly reduce maize yields (Nelly et al., 2022).

However, despite the recognized threat posed by *S. pacificus*, limited research has been conducted on the morphological and molecular identification of this species, particularly in Indonesia (Yashiro & Sanada-Morimura, 2022). Most of the existing studies focus on general pest control (Susilo et al., 2017; Nelly et al., 2017), while specific identification efforts—especially those utilizing molecular tools such as mitochondrial Cytochrome Oxidase I (mtCOI) DNA analysis—remain scarce. This gap in knowledge creates challenges in developing effective pest management strategies. Thus, further research is crucial to provide more comprehensive insights into the characteristics of this pest, facilitating better control methods.

This study aims to fill this gap by identifying *S. pacificus* on maize crops using both morphological and molecular approaches, with a focus on mtCOI DNA analysis. Accurate, rapid, and precise identification of this pest is crucial to enable effective control measures. This research is expected to contribute to a better understanding of the morphological and molecular characteristics of *S. pacificus* and support the development of more targeted and effective pest control strategies to prevent extensive damage to maize crops in the future.

MATERIALS & METHODS

Study location

Lamongan Regency, Indonesia, was selected as the sampling site because it ranks as the top food-producing region in East Java and is one of the top five food-producing regions nationally (Damayanti et al., 2023). According to BPS (2019), the three selected sub-districts have high maize production: Ngimbang (46,704 tons/ha), Sambeng (74,062 tons/ha), and Mantup (69,840 tons/ha).

Sample collection

This research was conducted from January to April 2024. *S. pacificus* specimens were collected from maize fields in the three sub-districts of Lamongan Regency, Indonesia, i.e., Ngimbang, Sambeng, and Mantup (see Figure 1). Samples were preserved in tubes containing 70% alcohol for further analysis. The samples were collected from maize plants in the vegetative stage. Morphological identification of the maize planthopper was conducted at the Plant Health Laboratory of the Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, while molecular identification was carried out at PT Genetika Science Indonesia.

Morphological identification

S. pacificus was identified at the species level by comparing the specimens to references from books and other relevant literature. The primary sources used were Borror et al. (2005), Gibb and Oseto (2020), and Bartlett et al. (2014). Additionally, related journal articles on maize planthoppers were also referenced.

DNA extraction

DNA was extracted using the gSYNC™ DNA Extraction Kit (Geneaid GS300, Taiwan). The extraction process followed three main principles: cell wall disruption, DNA separation from other cellular components, and DNA purification. Mitochondrial DNA (mtDNA) was selected for analysis due to its frequent use in phylogenetic studies of both invertebrates and vertebrates, with the Cytochrome Oxidase I (COI) gene being a reliable genetic marker (Maramis et al., 2014).

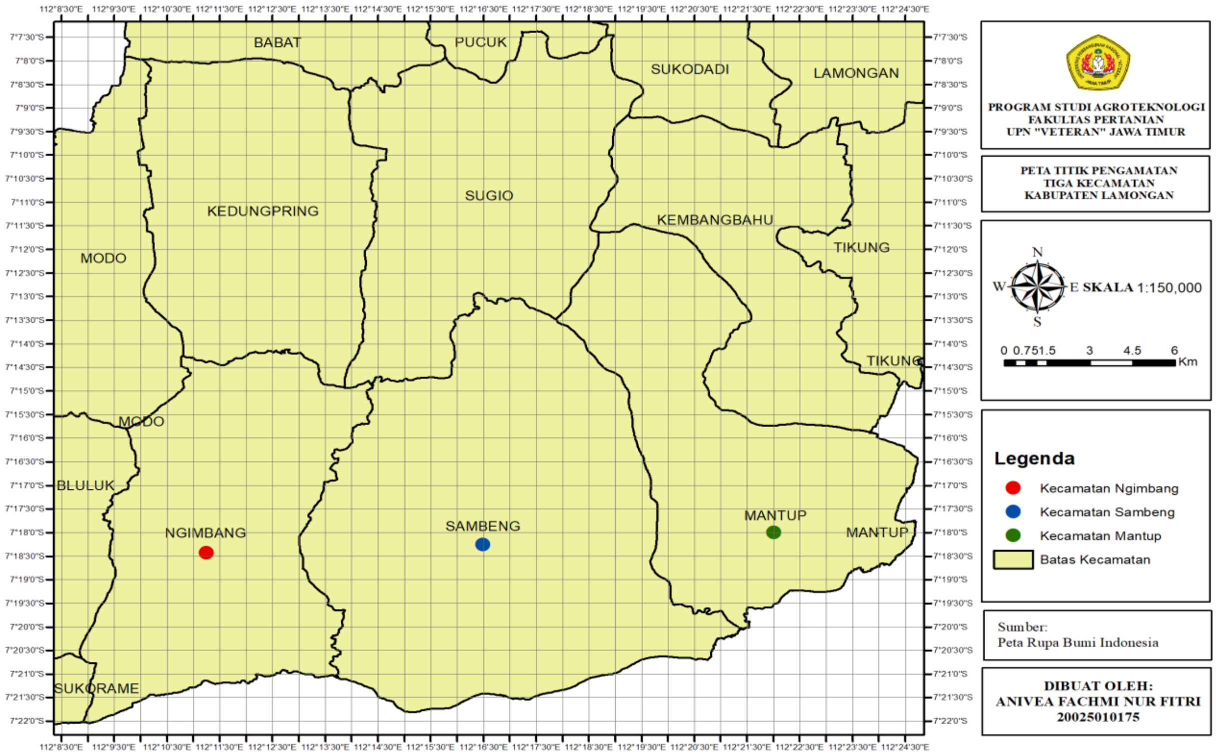

Figure 1. Sampling locations for maize planthoppers in Ngimbang, Sambeng, and Mantup sub-districts. This map was created using ArcMap (Environmental Systems Research Institute, Inc., USA).

Table 1. PCR cycle conditions for *S. pacificus* DNA amplification

Stage	Temperature (°C)	Duration	Cycles
Initial denaturation	95	1 min	1
Denaturation	95	10 s	-
Annealing	52	15 s	35
Extension	72	15 s	-
Final Hold	4	∞	1

DNA amplification

DNA amplification was performed using MyTaq HS Red Mix (Bioline BIO-25048, USA). The amplification process used primers LCO1490 (5′-GGTCAACAAATCATAAAGATAATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). The PCR amplification followed several cycles, and the amplified product was subsequently analyzed through electrophoresis on a 1% agarose gel. Visualization was carried out under UV light to observe the amplified DNA fragment (see Table 1).

Phylogenetic analysis

Phylogenetic analysis was performed using the nucleotide sequence of the COI gene, processed with the MEGA 11 software (USA) using the Maximum Likelihood method. This method measures the differences between nucleotide sequences or amino acids to assess genetic similarity. The smaller the differences, the closer the genetic relationship between the compared species (Yuniarti et al., 2016). The analysis was used to determine the evolutionary relationships between the *S. pacificus* samples and other related species, as well as to construct a phylogenetic tree.

RESULTS & DISCUSSION

Morphological identification of S. pacificus

Morphological identification, also known as conventional identification, involves direct observation of physical characteristics and comparing them to insect identification keys and taxonomic references (Indriati & Hidayat, 2023). The results from this study confirmed that the planthopper species found in the maize fields was *S. pacificus*, commonly referred to as the white-bellied planthopper (Susilo et al., 2017), as shown in Figure 2. This species was found across all sampled fields and exhibited a uniform distribution pattern. Similarly, Nelly et al. (2017) reported a uniform distribution of *S. pacificus* during the vegetative stage of maize in Pasaman Barat, Indonesia.

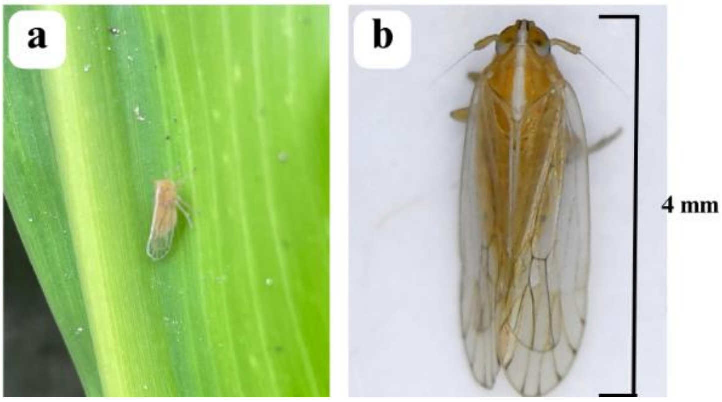

Figure 2. *S. pacificus* maize planthopper: (a) in the field; (b) under the microscope.

S. pacificus is a phytophagous insect that derives nutrients by feeding on the phloem sap of plant tissues. Although it has not yet been reported as a vector for plant pathogens, its feeding activity can significantly weaken the plant, leading to chlorosis, necrosis, and stunted growth (Hasibuan et al., 2021). The key morphological features distinguishing *S. pacificus* include the orange abdomen in male imagos and the white abdomen in females (Figure 3). These findings are consistent with previous studies by Hasibuan et al. (2021), which noted that female *S. pacificus* imagos possess a white waxy layer on the abdomen, while male imagos lack this layer and have an orange abdomen. Simbolon et al. (2020) further stated that male and female imagos can be distinguished by their abdominal characteristics, where female imagos have a white waxy substance on the lower abdomen, while male imagos do not have the waxy substance but instead exhibit a bright orange lower abdomen.

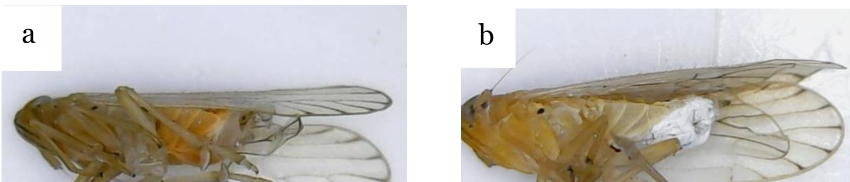

Figure 3. *S. pacificus* maize planthopper: (a) male imago abdomen; (b) female imago abdomen.

Additionally, *S. pacificus* has a light brown mesonotum and a distinctive movable spur at the end of the hind tibia, characteristic of the Delphacidae family (Susilo et al., 2017). The morphological traits observed in this study align with previous descriptions by Simbolon et al. (2020), who noted the absence of lateral spines on the first tarsal segment and a non-U-shaped pygofer diaphragm in male imagos.

Molecular identification of S. pacificus

Molecular identification was performed using mitochondrial Cytochrome Oxidase I (mtCOI) DNA, which is widely accepted for phylogenetic studies and species identification due to its universal accuracy (Maramis et al., 2014). The PCR amplification of the mtCOI gene yielded a fragment approximately 684 base pairs (bp) in length, as visualized on a 1% agarose gel (Figure 4). This fragment size is consistent with previous studies that have used the COI gene for insect identification (Darupamenang et al., 2022).

Table 2. BLAST results of DNA polymerase using nucleotide sequences.

Description	Max Score	Total Score	Query Cover	E. value	Per. Ident	Accession
S. pacificus WRJJ_UNILA	1164	1164	93%	0.0	99.53%	LC412751.1
S. pacificus WRJB_UNILA	1164	1164	93%	0.0	99.53%	LC412749.1
S. pacificus isolate SP9	1074	1074	89%	0.0	98.21%	MZ883125.1
S. pacificus isolate SP4	1074	1074	89%	0.0	98.21%	MZ883120.1
S. pacificus isolate SP24	1068	1068	89%	0.0	98.05%	MZ883140.1
S. pacificus isolate SP23	1068	1068	89%	0.0	98.05%	MZ883139.1
S. pacificus isolate SP22	1068	1068	89%	0.0	98.05%	MZ883138.1
S. pacificus isolate SP21	1068	1068	89%	0.0	98.05%	MZ883137.1
S. pacificus isolate SP20	1068	1068	89%	0.0	98.05%	MZ883136.1
S. pacificus isolate SP19	1068	1068	89%	0.0	98.05%	MZ883135.1
S. pacificus isolate SP18	1068	1068	89%	0.0	98.05%	MZ883134.1
S. pacificus isolate SP17	1068	1068	89%	0.0	98.05%	MZ883133.1
S. pacificus isolate SP16	1068	1068	89%	0.0	98.05%	MZ883132.1
S. pacificus isolate SP15	1068	1068	89%	0.0	98.05%	MZ883131.1
S. pacificus isolate SP14	1068	1068	89%	0.0	98.05%	MZ883130.1
S. pacificus isolate SP13	1068	1068	89%	0.0	98.05%	MZ883129.1
S. pacificus isolate SP12	1068	1068	89%	0.0	98.05%	MZ883128.1
S. pacificus isolate SP11	1068	1068	89%	0.0	98.05%	MZ883127.1
S. pacificus isolate SP10	1068	1068	89%	0.0	98.05%	MZ883126.1
S. pacificus isolate SP8	1068	1068	89%	0.0	98.05%	MZ883124.1
S. pacificus isolate SP7	1068	1068	89%	0.0	98.05%	MZ883123.1
S. pacificus isolate SP6	1068	1068	89%	0.0	98.05%	MZ883122.1
S. pacificus isolate SP5	1068	1068	89%	0.0	98.05%	MZ883121.1
S. pacificus isolate SP3	1068	1068	89%	0.0	98.05%	MZ883119.1
S. pacificus isolate SP2	1068	1068	89%	0.0	98.05%	MZ883118.1
S. pacificus isolate SP1	1068	1068	89%	0.0	98.05%	MZ883117.1

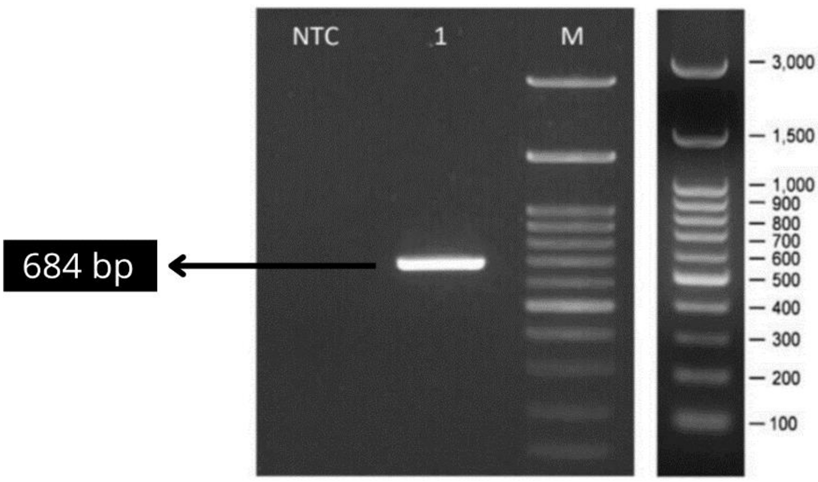

Figure 4. PCR DNA fragment of *S. pacificus* electrophoresed on agarose gel, visualized through UV transillumimator.

Figure 4 shows a clear and bright DNA fragment band, which indicates high-quality DNA suitable for sequencing. A sharp band confirms that the DNA is intact and ready for further sequencing analysis. In contrast, lower-quality bands or smeared results typically suggest contamination or improper amplification (Jamaluddin et al., 2024). However, even if the band appears less prominent, the amplification process can still be considered successful as long as there is no smear or tail observed in the electrophoresis results (Mawardi et al., 2020).

The amplified DNA was sequenced and analyzed using the Basic Local Alignment Search Tool (BLAST) program with the database from the National Center for Biotechnology Information (NCBI). This analysis compares the DNA sequences of the samples with those registered in the database, providing information that verifies the similarity of the sample's DNA sequence with other insects listed in the database, thus facilitating identification. Kusumah et al. (2022) stated that phylogenetic relationships between species can be determined through BLAST analysis, which is preceded by the alignment of nucleotide and amino acid sequences using specific software. The BLAST results revealed a 99.53% similarity between the *S. pacificus* sample (SP_CO1) and the *S. pacificus* WRJJ_UNILA and WRJB_UNILA mitochondrial COI gene for cytochrome c oxidase subunit I, both of which were isolated in Indonesia (Table 2). This high degree of similarity confirms the identity of the planthopper as *S. pacificus*. The BLAST analysis also indicated alignment with other *S. pacificus* isolates registered in the NCBI database, with slightly lower similarity percentages (98.05%–98.21%) for other isolates (see Table 2).

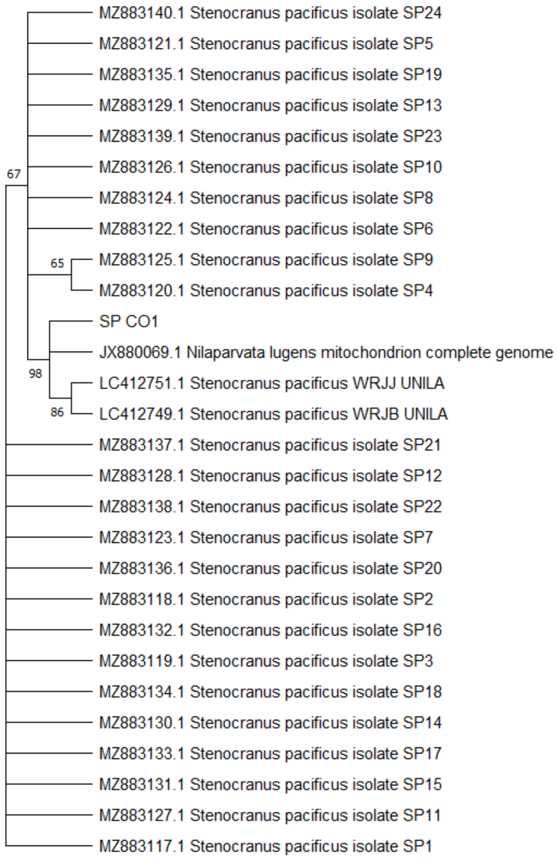

Figure 5. Phylogenetic tree of *S. pacificus* based on mtCOI DNA fragment sequences using MEGA 11 software.

Phylogenetic analysis

The phylogenetic tree constructed from the COI gene sequence using the Maximum Likelihood method in MEGA 11 software (Figure 5) supports the findings from the BLAST analysis. The tree shows that the *S. pacificus* sample (SP_CO1) is closely related to *S. pacificus* WRJJ_UNILA and WRJB_UNILA, with a similarity of 99.53%. The evolutionary distance between the sequences is minimal, indicating that they share a recent common ancestor (Fietri et al., 2021). Figure 5 illustrates the evolutionary relationships between the different *S. pacificus* isolates, where the short branch lengths indicate close genetic relationships, as previously described by Oktafia and Badruzsaufari (2021). The longer the branch, the greater the evolutionary divergence between species. In this case, the short branch lengths suggest that the *S. pacificus* isolates share a recent common ancestor and are genetically similar, supporting the hypothesis that the samples from Lamongan are indeed *S. pacificus*.

The identification of *S. pacificus* through both morphological and molecular methods highlights the importance of accurate and timely pest identification. Effective pest control measures can only be implemented if the species is properly identified. Hasibuan et al. (2021) emphasized that accurate identification is crucial for the development of pest management strategies, as it allows for targeted interventions that can mitigate crop damage. This study contributes valuable insights into the morphological and molecular characteristics of *S. pacificus*, providing a foundation for future research on pest control strategies. By combining conventional morphological methods with molecular techniques, this research enhances our understanding of *S. pacificus*, enabling more precise and effective control measures.

CONCLUSIONS

This study successfully identified *S. pacificus* as a primary maize pest in the sampled agricultural areas using both morphological and molecular methods. The morphological identification revealed key distinguishing features, such as the orange abdomen in male imagos and the white abdomen in female imagos, consistent with previous reports on *S. pacificus*. Molecular analysis, using mitochondrial Cytochrome Oxidase I (mtCOI) DNA, confirmed a 99.53% similarity between the *S. pacificus* specimens from Lamongan and previously documented isolates from Indonesia. The phylogenetic analysis further supported the close genetic relationship between the sampled *S. pacificus* and other related species. These findings highlight the utility of combining traditional morphological techniques with molecular tools for accurate and rapid pest identification, which is crucial for developing effective pest management strategies. In conclusion, this study provides valuable insights into the morphological and molecular characteristics of *S. pacificus*, offering a strong foundation for future research. The ability to accurately identify *S. pacificus* will aid in the timely implementation of control measures, thereby mitigating the potential damage caused by this pest to maize crops. Future studies should focus on exploring the ecological impact of *S. pacificus* and evaluating integrated pest management strategies to reduce its threat to maize production.

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