



Submissions for Diagnostic Protocols

I. General information

Submission number	2023-016
Title of Proposal	Diagnostic protocol for <i>Bactrocera tsuneonis</i> and <i>Bactrocera minax</i>
Submitted by (Country or Organization)	IPPC Contracting Party
IPPC Official Contact Point or RPPO	China
Supported by	China Agricultural University

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	<p><i>Bactrocera tsuneonis</i> (Miyake, 1919) and <i>Bactrocera minax</i> (Enderlein, 1920) are economically important fruit flies of international concern, causing severe damage to citrus industries. Currently, there is no International Standards for Phytosanitary Measures (ISPM) specifically addressing the identification of these sibling fruit flies. Developing such a standard based on morphological and molecular diagnostic methods would effectively address the requirement for accurate and rapid identification of <i>B. tsuneonis</i> and <i>B. minax</i>, and provide a technical basis for their quarantine and identification. China Agricultural University, in collaboration with the other organizations, has developed morphological and molecular identification techniques for <i>B. tsuneonis</i> and <i>B. minax</i> which including DNA barcoding, PCR, LAMP and microfluidic dynamic array. Relevant papers have been published, and these techniques have been extensively applied. It is recommended to establish this standard to provide technical support for global management of these two fruit flies and facilitate trade of citrus.</p>
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Proposed priority	I (high)
Comments	The proposal highlights the issues of citrus production and international trade caused by <i>Bactrocera tsuneonis</i> and <i>Bactrocera minax</i> and emphasizes the urgent requirement for an internationally recognized diagnostic protocol. The collaborative efforts of China Agricultural University and other organizations, along with the successful development and application of morphological and molecular identification techniques, demonstrate the readiness and capability to contribute to the establishment of a comprehensive and effective standard. The proposed priority ranking of "Level I" reflects the significance and immediate attention this matter deserves in order to protect the global citrus industry and ensure the safe trade of produce.

4. Literature review

Literature review	<p><i>Bactrocera tsuneonis</i> and <i>Bactrocera minax</i> are two sibling species of the subgenus <i>Tetradacus</i> of <i>Bactrocera</i> (White and Elson-Harris, 1992). These key species hinder the citrus trade, cause significant financial losses, drastically lower citrus production and quality. <i>B. tsuneonis</i> and <i>B. minax</i> are very similar in all life stages. There are limited morphological characteristics to differentiate the adult species, and it is nearly impossible to differentiate these two species in the egg, larva, pupa stages. In recent decade, China Agricultural University and related organizations have developed the molecular diagnostic methods such as DNA barcoding (Jiang et al, 2014a), PCR (Jiang et al, 2014b; Zheng et al, 2019), LAMP (Yang et al, 2023) and microfluidic dynamic array techniques (Jiang et al, 2016) which published in papers. In PCR, the new species-specific markers were designed to identify these two species (Zheng, et al, 2019) and improve upon the false-positive results of previous species-detection primers (Jiang et al, 2014b). In LAMP method (Yang et al, 2023), two sets of primers were designed to distinguish <i>B. minax</i> and <i>B. tsuneonis</i> using DNA barcoding region of the COI gene, and only 50 min was needed under a constant temperature of 65 °C to determine the species of the two flies. The reaction system has high specificity and sensitivity, in which these two species can be accurately distinguished between different geographical populations and 1.0 ng/μL was the lowest DNA concentration that could be detected. In China, the morphological and molecular diagnostic methods have been applied for adults, larvae, pupae, eggs of <i>B. tsuneonis</i> and <i>B. minax</i> in recent five years. The main reference are as follows: (* corresponding author) Jiang, F., Jin, Q., Liang, L., Zhang, A.B.*, and Li, Z.H.* (2014a). Existence of species complex largely reduced barcoding success for invasive species of Tephritidae: a case study in <i>Bactrocera</i> spp. <i>Molecular Ecology Resource</i>, 14, 1114–1128. 10.1111/1755-0998.12259.</p> <p>Jiang, F., Li Z. H.*, Wu J. J., Wang F. X., Xiong H. L.. (2014b). A</p>
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	<p>rapid diagnostic tool for two species of <i>Tetradacus</i> (Diptera: Tephritidae: <i>Bactrocera</i>) based on species-specific PCR. <i>Journal of Applied Entomology</i>, 138: 418–422.</p> <p>Jiang, F., Fu, W., Clarke, A.R., Schutze, M.K., Susanto, A., Zhu, S.F.*, and Li, Z.H.* (2016). A high-throughput detection method for invasive fruit fly (Diptera: Tephritidae) species based on microfluidic dynamic array. <i>Molecular Ecology Resource</i>, 16, 1378–1388. 10.1111/1755-0998.12542.</p> <p>White I.M. and Elson-Harris M.M. (1992). <i>Fruit flies of economic significance: their identification and bionomics</i>, Wallingford, UK: CABI.</p> <p>Yang W.Z., Su Y., Zeng L.Y., Zhang Y., Ullah F., Wang X.L., Li X.N., Feng X.D., Li Z.H.*. (2023). LAMP assay as a rapid identification technique of Chinese citrus fly and Japanese orange fly (Diptera: Tephritidae). <i>Journal of Economic Entomology</i>, https://doi.org/10.1093/jee/toad014.</p> <p>Zheng L.Y., Zhang Y., Yang W.Z., Zeng Y.Y., Jiang F., Qin Y.J., Zhang J.F., Jiang Z.C., Hu W.Z., Guo D.J., Wan J., Zhao Z.H., Liu L.J., Li Z.H.*. New species-specific primers for molecular diagnosis of <i>Bactrocera minax</i> and <i>Bactrocera tsuneonis</i> (Diptera: Tephritidae) in China Based on DNA Barcodes. (2019). <i>Insects</i>, 10, 447; doi:10.3390/insects10120447</p>
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5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	<p><i>Bactrocera tsuneonis</i> and <i>Bactrocera minax</i> are economically important fruit flies causing issue to citrus production and international trade. These two species are very similar in all life stages. There are limited morphological characteristics to differentiate the adult species, and it is nearly impossible to differentiate these two species in the egg, larva, pupa stages. In recent decade, the morphological and molecular identification techniques for <i>B. tsuneonis</i> and <i>B. minax</i> has been studied and applied. The molecular diagnostic techniques include DNA barcoding, PCR, LAMP and microfluidic dynamic array. It is very necessary to establish this standard to provide technical support for global management of these two fruit flies and facilitate trade of citrus. Developing such a standard would effectively address the requirement for accurate and rapid identification of <i>B. tsuneonis</i> and <i>B. minax</i>, and provide a technical basis for their quarantine and identification.</p>
2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.	<p>The relevance of diagnosis to the protection of plants lies in its vital role in mitigating the impact of <i>B. tsuneonis</i> and <i>B. minax</i> and implementing effective measures for plant protection. Accurate diagnosis enables the early detection and identification of these two sibling species, facilitating timely and targeted interventions to control their spread and minimize damage to citrus production. Furthermore, diagnosis plays a crucial role in quarantine and biosecurity measures. It allows for the identification of <i>B. tsuneonis</i> and <i>B. minax</i>, enabling the implementation of strict inspection, test, treatment, and</p>

	surveillance to prevent their establishment and spread into new areas.
3. Importance of the plants protected on the global level (e.g. relevant to many countries or of major importance to a few countries).	<i>Bactrocera tsuneonis</i> (common name is Japanese orange fly) and <i>Bactrocera minax</i> (common name is Chinese citrus fly) are native to the East Asia region. <i>B. minax</i> is native to China and distributes in part of China, Bhutan and India till now. <i>B. tsuneonis</i> is native to Japan and distributes in a few regions in China and Japan. However, many countries in the world which have citrus production pay more attention on the prevention of these two key species and list them as quarantine pests. Thus, the importance of prevention and control of <i>B. tsuneonis</i> and <i>B. minax</i> for the plants protected is on the global level.
4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).	The international trade of citrus has been paid high attention by many countries in the world. <i>Bactrocera tsuneonis</i> and <i>Bactrocera minax</i> are economically important fruit flies causing severe damage to citrus industries and severe impact to citrus trades. The eggs, larvae and pupae of these two species can be spread in the world through the international fruits trade if we could not find them in time. With the diagnostic procedures based on molecular diagnosis particularly, we can effectively address the requirement for accurate and rapid identification of <i>B. tsuneonis</i> and <i>B. minax</i> . And it is the basis for further phytosanitary treatment and citrus trade.
5. Other criteria for topics as determined by CPM that are relevant to determining priorities	ISPM 27 Diagnostic protocols for regulated pests DP 09: Genus <i>Anastrepha</i> Schiner, DP 29: <i>Bactrocera dorsalis</i>
6. The balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.	According to the currently distribution of <i>B. tsuneonis</i> and <i>B. minax</i> , the climatic zones include tropical region, subtropical region and warm temperate zone. Although the distributions of two fruit flies are limit, their potential geographical distributions in the world are relatively wide. The larvae of <i>B. tsuneonis</i> and <i>B. minax</i> damage the citrus (e.g., orange, tangerine) segments and seeds, and they can move inside the fruit freely, causing internal decay of citrus. Immature fruit will turn from green to yellow, and then rot or lose moisture. These pests severely reduce the yield and quality of citrus and significantly suppress farmer's enthusiasm for planting citrus. Numerous citrus fruits could not be sold and rotted in the field or warehouse because consumers felt panic and disgust when they bought the fruit with larvae inside, which caused severe economic losses. We should have the prevention on the ports particularly for pest free areas and have the control in the citrus orchards particularly for pest area of <i>B. tsuneonis</i> and <i>B. minax</i> .
7. Number of labs undertaking the diagnosis.	Most laboratories of NPPOs are able to undertake the diagnosis of <i>B. tsuneonis</i> and <i>B. minax</i> , as it includes morphological and molecular identification methods. The DNA barcoding, PCR, and LAMP mentioned in the protocol is suitable for application in the vast majority of national and regional laboratories, and have very high application value.

<p>8. Feasibility of production of a protocol, including availability of knowledge and expertise.</p>	<p>(1) Knowledge and Expertise: The studies demonstrate a solid understanding of the genetic markers and detection methods for <i>Bactrocera tsuneonis</i> and <i>Bactrocera minax</i>. The authors (particularly from China Agricultura University) have expertise in the field of entomology, genetic analysis, and molecular biology, which is essential for developing an execution standard.</p> <p>(2) Existing Research: The referenced articles provide a foundation for the development of an execution standard by outlining morphological and molecular diagnosis for <i>B. tsuneonis</i> and <i>B. minax</i> which includes DNA barcoding, PCR and LAMP etc. These studies contribute to the existing knowledge and can serve as a basis for the development of standardized protocols.</p> <p>(3) Relevance and Importance: The economic significance of <i>B. tsuneonis</i> and <i>B. minax</i> highlight the need for an execution standard to facilitate accurate and efficient identification and monitoring. This further emphasizes the relevance and importance of developing a standardized protocol for <i>B. tsuneonis</i> and <i>B. minax</i>.</p> <p>(4) Practical Implementation: In recent decade, the morphological and molecular identification techniques for <i>B. tsuneonis</i> and <i>B. minax</i> has been applied in China and other countries. These techniques can potentially be translated into an execution standard that provides specific instructions and guidelines for practitioners in the field. Based on the above analysis, it appears feasible to develop an execution standard for the rapid detection and identification of <i>B. tsuneonis</i> and <i>B. minax</i>. The existing knowledge, expertise, and practical implementation methods described in the referenced studies provide a strong foundation for the development of standardized protocols in this area.</p>
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