Food and Agriculture Organization of the United Nations

International Plant Protection Convention

REPORT

Technical Panel on Diagnostic Protocols (TPDP)

Virtual Meeting
7 March 2023

IPPC Secretariat

Required citation:

IPPC Secretariat. 2023. *Report of the virtual meeting of the Technical Panel on Diagnostic Protocols (TPDP), 7 March 2023.* Rome. Published by FAO on behalf of the Secretariat of the International Plant Protection Convention (IPPC).

CONTENTS

1. Opening of the meeting

1.1 Welcome by the IPPC Secretariat

[1] On behalf of the secretariat of the International Plant Protection Convention (IPPC) (hereafter referred to as "the secretariat"), Adriana MOREIRA welcomed participants to this meeting of the Technical Panel on Diagnostic Protocols (TPDP), which was being held in virtual mode.

2. Meeting arrangements

2.1 Selection of chairperson

[2] The TPDP selected Norman BARR (United States of America) as chairperson.

2.2 Selection of the rapporteur

[3] The TPDP selected Vessela Assenova MAVRODIEVA (United States of America) as rapporteur.

2.3 Adoption of the agenda

[4] The TPDP adopted the agenda (Appendix 1).

3. Administrative matters

[5] The secretariat informed the TPDP that Robert TAYLOR (New Zealand), Yazmin RIVERA (United States of America) and Liping YIN (China) could not attend the meeting.

[6] The TPDP welcomed the new panel member, Andrew Sarkodie APPIAH (Ghana).

4. Recommendation to the SC for adoption

4.1 Revision and approval of *Mononychelus tanajoa* (2018-006)

[7] The Discipline Lead Juliet GOLDSMITH (Caribbean Agricultural Health and Food Safety Agency) introduced the draft diagnostic protocol (DP) for *Mononychelus tanajoa* (2018-006), together with supporting documentation.[1] The draft DP had been revised by the drafting group since the TPDP's meeting in October–November 2022 and the TPDP was now invited to review it and consider recommending it to the SC for approval for adoption.

[8] The discipline lead explained that, at the TPDP's meeting in October–November 2022, the TPDP had addressed most of the issues with the draft DP. However, there had been 12 issues arising from consultation or subsequent TPDP discussion that required further information or clarification from the DP authors. She had therefore sought feedback on these issues from the authors. The discipline lead presented the authors' responses and the TPDP considered them.

[9] **Pest information.** For this section, the discipline lead had checked with the authors whether they had a reference to overwintering of *M. tanajoa* in temperate climates and, if not, whether it would be acceptable to delete any mention of temperate climates. The authors had agreed to the deletion, and the TPDP supported this.

[10] **Detection.** One consultation comment had queried the phrase "adult females and males are used for identification with dichotomous keys" in this section, as it was not clear whether the species could be identified from an adult female or an adult male using the key or whether both are needed. The authors had confirmed that the intended meaning was the latter and they had therefore accepted the editorial

[1] 2018-006; 02_TPDP_Tel_2023_Mar; 03_TPDP_Tel_2023_Mar; 04_TPDP_Tel_2023_Mar.

change made by the TPDP in Paris: "both female and male adults are needed for identification with dichotomous keys".

[11] **Preparation of specimens for microscopic examination.** The TPDP reviewed the authors' responses to questions about the concentration of lactic acid to be used, whether to present maceration as a separate step, and the temperature at which to heat mounted slides.

[12] The authors had confirmed that the lactic acid concentration for clearing should be 60–95% and the TPDP agreed. The TPDP noted that the concentration for mounting (in Hoyer's medium) should remain as 85–92%.

[13] The discipline lead noted that maceration, which is useful for mites that are sclerotized, is part of the clearing step as it uses the same solution used for clearing and is done at the same time. She had therefore referred to maceration in the text but not described it as a separate step. The authors had agreed to the editorial changes and the TPDP agreed.

[14] The authors had confirmed that, based on their experience, freshly mounted slide specimens (in Hoyer's medium) could be kept on a hot plate at a constant temperature of 70 °C for at least 24 hours without any problems. They suggested, however, that the time period for heating be described as "20 minutes" rather than "at least 20 minutes". In addition, they agreed that the temperature of 40–45 °C proposed in a consultation comment would also work but the heating time would need to be much longer. The TPDP therefore agreed that the heating before identification should be specified as "70 °C for 20 minutes" and that 40–45 °C should be used when drying slides to be stored.

[15] **Morphological characters of the family Tetranychidae.** The discipline lead explained that the authors had provided an additional image to illustrate whip-like movable digits, and this was now cross-referenced from the text in this section. She clarified that many of the figure numbers in the draft DP had changed in the current draft, so no longer matched the figure numbers in the consultation comments.

[16] **Dichotomous key to genera of Tetranychidae on *Manihot* spp.** The discipline lead explained that the authors had provided an image of *Allonychus* sp. to illustrate the empodial claw being shorter than the proximoventral hairs, and this was cross-referenced from the relevant couplet of this key.

[17] **Morpohological identification of *Mononychellus tanajoa*.** The discipline lead explained that, in response to the consultation comment suggesting that a numerical range be given for the length and width of the idiosoma in adult males, rather than a single value for each, the authors had agreed and confirmed that the appropriate ranges were 275–308 µm for the length and 167–178 µm for the width. They had also requested that the width range given for the idiosoma of females be changed from 275–335 µm to 187–217 µm. The TPDP agreed and the discipline lead undertook to make this change.

Primers for conventional polymerase chain (PCR) reaction and sequencing of *Mononychellus tanajoa*. One consultation comment had suggested that further explanation be given for the statement in the draft DP that the primers of Folmer *et al*. (1994) may not always work for *Tetranychus* spp. and that, if this is the case, the primers of Li *et al*. (2015) can be used for amplification.[2] The TPDP acknowledged that the authors' response to the query about this had not resolved the fundamental question of why both sets of primers are needed in the protocol. One TPDP member, however, had investigated the matter in more detail since the last TPDP meeting and provided the following supplementary information:

- the primers of Folmer *et al.* (1994) amplify the DNA segment that corresponds to the reference sequence on GenBank for this species, so can support identification, whereas the segment amplified by the primers of Li *et al*. (2015) does not completely coincide with that amplified by the primers of Folmer *et al.* (1994) and does not encompass all the barcoding fragment of the *COI* gene;
- the reference sequence on GenBank, which is the only reference on GenBank for this species, was obtained using the Folmer *et al.* (1994) primers;

[2] For references, see Appendix 2.

- the theoretical maximum coverage that could be obtained using the primers of Li *et al*. (2015) is 80%, which is far short of the 90% query cover required by the protocol to confirm species-level identification; and
- although the current text could imply that the primers of Li *et al*. (2015) always worked and those of Folmer *et al.* (1994) did not, the primers of Li *et al*. (2015) also did not work all of the time.

[18] Although the authors had explained that, in their laboratory experience, the primer set of Folmer *et al.* (1994) sometimes does not work well for *Tetranychus* spp., the TPDP agreed that the protocol could not be based on such evidence alone, as it needed to be validated.

[19] The TPDP noted that, in the current draft of the protocol, molecular methods provided information to support diagnosis rather than to confirm it; morphological examination of adults was always required to achieve identification. So, it was not essential to give a second set of primers for use when the Folmer *et al.* (1994) primers did not work.

[20] In the light of their discussion, the TPDP agreed to the remove the Li *et al*. (2015) primers from the protocol, noting that although the protocol would then only specify use of the Folmer *et al.* (1994) primers, this would not prevent laboratories from validating other methods themselves.

[21] The TPDP agreed that the response to the relevant consultation comment would need updating to explain that the Folmer *et al.* (1994) primers have been shown to work well with the target species for this protocol and they generate the data most equivalent to the sequence used for diagnosis in this protocol, whereas the primers specified in Li *et al*. (2015) would not add any information of value when identifying this target species.

[22] **Expected amplicon size for *Mononychellus tanajoa*.** Further to a query raised by the TPDP, the discipline lead had asked the authors to confirm the expected amplicon size, as the PCR table in the protocol gave a size of 600–650 base pairs (bp), citing Ovalle *et al*. (2020), but the GenBank reference sequence was 597 bp. The authors had confirmed that the amplicon presented in Ovale *et al*. (2020) is around 600 bp, but that the size of the DNA fragment visualized in the gel can vary slightly according to the gel imaging system used and the actual size of the DNA fragment is as per the sequence on GenBank. The TPDP noted that the 597 bp size given on GenBank is likely to be the size of the fragment after the primers and the bases close to the primers have been trimmed off (i.e. the functional sequence), whereas the size to be given in the PCR table was the size of the amplified product (i.e. before trimming). One of the TPDP members noted that a range of 600–650 bp was commonly cited in the entomological literature, but the member had calculated that the amplified product in this case should be 709 bp, based on the complete mitochondrial genome data for *Panonychus citri* (GenBank accession number NC_014347). The TPDP therefore agreed to give the expected size of the amplicon as 709 bp rather than 600–650 bp.

[23] **Sequence editing and analysis.** The discipline lead explained that she had asked the authors to consider referring to studies supporting the 97% similarity threshold for species-level identification specified in the protocol. The authors had responded by agreeing with the corresponding consultation comment, which had said that a 99% similarity is much more reliable, particularly considering that just one sequence is available on GenBank. However, the authors had not suggested how to adjust the draft DP (i.e. whether to change the percentage similarity to 99%).

[24] The TPDP noted that a molecular determination just provides information to support a diagnosis made by morphological examination, so it is not essential. However, they recognized that if the target species is identified based on morphology but the molecular method shows a similarity of 96%, this may then give rise to a dilemma for the national plant protection organization (NPPO) on how to interpret the results.

[25] The TPDP agreed that the main question was what percentage similarity would provide NPPOs with sufficient confidence to make quarantine decisions. One TPDP member commented that, whatever the percentage-similarity threshold used, a reference for it should be given in the draft DP. Another member

suggested that the authors be asked whether they have data on the species demarcation criteria for a species that is closely related to *M. tanajoa*.

[26] The TPDP agreed that the discipline lead would liaise with the authors for further information and recommendations, and the TPDP would then decide by email the changes to be made to the draft DP.

[27] **Nomenclature for setae.** The discipline lead confirmed that, upon request, the authors had provided a reference for the nomenclature used to name setae and this had been included in the draft DP.

[28] **Figure showing lateral view of adult male aedeagus of *M. tanajoa*.** The discipline lead referred to the consultation comment that that had queried whether the photograph and line drawing in this figure are definitely of *M. tanajoa*, as although the images were consistent with the description in the reference cited, they differed from illustrations in another source, Gutierrez (1987). The authors had explained that both the original description of the species and subsequent relevant publications had provided insufficient taxonomic information on the shape of the male aedeagus, but a literature review had revealed two different types of aedeagi. Although Gutierrez (1987) had presented line drawings of the aedeagi of eight species of *Mononychellus* including *M. tanajoa*, they had not specified the collection details of the specimens used for the drawings and so it was impossible to verify the species identity. The authors had therefore suggested that the current images be retained in the draft DP, as these images were consistent with those in two other publications (for which they provided the references) and the source of the corresponding specimens was Brazil, which was where the type specimen of this species was found. The TPDP agreed.

[29] **Figure showing types of pretarsi in Tetranychidae on *Manihot* spp.** The discipline lead confirmed that, in response to a consultation comment, the authors had updated the caption to this figure and had provided a new image for it.

[30] The TPDP noted that, for this and some other figures, italics needed to be applied to species names, but they recognized that this could be dealt with during editing.

[31] **Labels for figures.** The secretariat highlighted the desirability, where possible, of not including embedded labels in images in case the DP is translated in the future, as translators would need to be able to convert the labels to the relevant language. They confirmed, however, that DPs are only translated when funds are available.

[32] **Next steps.** The secretariat confirmed that once the changes discussed at this meeting had been made, the draft DP would then pass to the editing stage, after which it would be submitted to the SC to approve for adoption. The draft DP would then be submitted to the DP notification period.

[33] For the benefit of new TPDP members, the chairperson recalled that it is permitted for the TPDP to make changes to the text of a draft DP even if the authors disagree, as DPs are collective works not just the sole work of the authors. He commented that, should this happen, it may be helpful for the TPDP to offer assistance to the discipline lead in drafting responses.

The TPDP:

(1) *thanked* the drafting group and the discipline lead for the updates made to the draft DP;

(2) *agreed* that the discipline lead would liaise with the authors regarding the percentage-similarity threshold to cite for species-level identification using PCR and that the TPDP would subsequently reach a conclusion on the percentage similarity by email;

(3) *agreed* that the discipline lead would revise the draft DP for *Mononychelus tanajoa* (2018-006) and the corresponding responses to comments as discussed at this meeting and according to the subsequent email conclusion on percentage similarity, and provide a final version to the secretariat; and

agreed with the responses to the comments and with the draft DP for *Mononychelus tanajoa* (2018-006) and *recommended* the draft DP to the SC for approval for adoption on behalf of the Commission on Phytosanitary Measures.

5. TPDP work programme

5.1 Updates and review of draft DPs in the work programme

[34] The TPDP reviewed the progress of DPs in the work programme.

[35] Two TPDP members had already sent updates on the draft DPs for which they were discipline lead. The secretariat suggested that all other discipline leads also send an update to the secretariat by e-mail.

The TPDP:

(4) *agreed* to provide updates to the secretariat on progress with the draft DPs for which they are discipline lead; and

(5) *requested* that the secretariat circulate the latest version of the TPDP work programme to all TPDP members.

5.2 Updates from the IPPC Secretariat

[36] The secretariat confirmed that the expert consultation on the revision of DP 25 (*Xylella fastidiosa*) had recently closed, with six comments received (some of which were merged comments from more than one source). The expert consultation on pospiviroid species had opened on 6 March with a deadline of 31 March, and one on the revision of DP 9 (Genus *Anastrepha*) would be opened very soon.

[37] The secretariat also confirmed that the report from the October–November 2022 TPDP meeting in Paris would be posted shortly and that the TPDP update to the SC was being prepared with the TPDP steward.

[38] Finally, the secretariat alerted the TPDP to the 2023 IPPC Call for Topics: Standards and Implementation[3] that would be opened around June 2023.

5.3 Action points from the November 2022 TPDP meeting

[39] The TPDP reviewed the action points from the TPDP' November 2022 meeting.[4]

[40] The secretariat confirmed that the various matters requiring input from the SC would be presented to the SC in an e-decision shortly after this meeting. The secretariat explained that the aim was to get as many draft DPs as possible through to the consultation stage this year, but there are limitations because of time constraints. However, suggestion to hold more than one consultation period in 2024 because of the number of draft DPs is to be presented once again to the SC, but this time for discussion.

6. Any other business

[41] The secretariat confirmed that the next face-to-face meeting has been tentatively scheduled for 28 August to 1 September 2023. It was premature to confirm the location, but it was likely to be in Europe.

7. Close of the meeting

[42] The chairperson thanked the TPDP and the secretariat and closed the meeting.

[3] IPPC Call for topics: standards and implementation: https://www.ippc.int/en/core-activities/standards-and-implementation/call-for-topics-standards-and-implementation/
[4] 05_TPDP_Tel_2023_Mar.

Appendix 1: Agenda

2023 MARCH VIRTUAL MEETING OF THE TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS (TPDP)

07 March 2023
12:00-14:00 (GMT+1)

AGENDA

Agenda Item		Document No.	Presenter
1.	**Opening of the Meeting**		
1.1	Welcome by the IPPC Secretariat	--	IPPC Secretariat (MOREIRA)
2.	**Meeting Arrangements**		
2.1	Selection of Chairperson	--	MOREIRA
2.2	Selection of the Rapporteur	--	Chairperson
2.3	Adoption of the Agenda	01_TPDP_Tel_2023_Mar	Chairperson
3.	**Administrative Matters**		
3.1	Participants / membership	TPDP membership list	MOREIRA / MONTEROSA
3.2	Connections to Zoom and virtual meetings	Short guideline for participants	
4.	**Recommendation to the SC: Review of draft diagnostic protocols (DPs) (from IPPC consultation period)**		
4.1	*Mononychelus tanajoa* (2018-006) Discipline lead: Juliet GOLDSMITH Referee: Norman BARR - Compiled comments - Responses to compiled comments - Summary of major comments received (as for the 2022-11 TPDP meeting) - Summary of TPDP discussions	2018-006 02_TPDP_Tel_2023_Mar 03_TPDP_Tel_2023_Mar 04_TPDP_Tel_2023_Mar	GOLDSMITH
5.	**TPDP work programme**		
5.1	Updates and review of draft DPs in the work programme	-	Chairperson / DLs
5.2	Updates from IPPC Secretariat	-	IPPC Secretariat
5.3	Action points from the 2022-11 TPDP meeting	05_TPDP_Tel_2023_Mar	Chairperson / IPPC Secretariat
6.	**Any other business**	-	Chairperson
7.	**Closing of the meeting** - Recommendations to SC or IPPC Secretariat	-	Chairperson

Appendix 2: References

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294–299.

Gutierrez, J. 1987. The cassava green mite in Africa: one or two species? (Acari: Tetranychidae). *Experimental & Applied Acarology*, 3: 163–168.

Li, D., Fan, Q.-H. Waite, D.W., Gunawardana, D., George, S. & Kumarasinghe, L. 2015. Development and validation of real-time PCR assay for rapid detection of two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *PLoS ONE*, 10(7): e0131887. https://doi.org/10.1371/journal.pone.0131887

Ovalle, T.M., Vásquez-Ordóñez, A.A., Jimenez, J., Parsa, S., Cuellar, W.J. & Lopez-Lavalle, L.A.B. 2020. A simple PCR-based method for the rapid and accurate identification of spider mites (Tetranychidae) on cassava. *Scientific Reports*, 10: 19496.