2024 FIRST CONSULTATION

30 January – 03 May 2024

Compiled comments for 2024 First Consultation:2021-015_DP_Heterobasidion

Summary

Participants

Name	Summary
Benin	Le Bénin remercie l'équipe de travail et prend note du contenu du projet Merci

T (Type) - B = Bullet, C = Comment, P = Proposed Change, R = Rating
S (Status) - A = Accepted, C = Closed, O = Open, W = Withdrawn, M = Merged

Para	Text	T	Comment
G	(General Comment)	C	*Category : SUBSTANTIVE* **(186) Barbados (3 May 2024 6:27 PM)** The protocol has been well written and the extensive list of references is a big plus. It is a good addition to the ISPM 27.
G	(General Comment)	C	*Category : EDITORIAL* **(172) Nepal (1 May 2024 1:41 PM)** Nepal does not have any comment on the draft annex
G	(General Comment)	C	*Category : SUBSTANTIVE* **(171) Philippines (1 May 2024 11:45 AM)** 1. NPPO to verify if H. annosum species complex present in the Philippines - Notably, there are no significant reports to support that its hosts Abies alba and Picea abies are present in the Philippines. Only Pinus sp. can be found in Benguet, thus naming it Benguet pine. 2. There are some reports that use serological tests for the detection of H. annosum sensu lato. Suggestion to clarify if this was feasible to be included as these are in the early phase.
G	(General Comment)	C	*Category : TECHNICAL* **(170) Benin (30 Apr 2024 11:54 AM)** Le Bénin n'a pas d'objection sur ce projet Merci
G	(General Comment)	C	*Category : TECHNICAL* **(169) EPPO (29 Apr 2024 5:57 PM)** There is only one thing I am slightly

			perplexed by and it has taken a little while to find the reference associated with it. Where they discuss using PCNB-based selective culture medium for isolation the protocol mentions to include the streptomycin in the initial mix prior to it being autoclaved. For an antibiotic I would have thought this would have degraded it to a point of it not being useful. I thought this may have been mixed up when the protocol was written so I checked the original paper : Kuhlman, E.G. & Hendrix Jr, F.F. 1962. A selective medium for the isolation of Fomes annosus. Phytopathology, 52: 1310–1312. where they do actually autoclave the antibiotic. I haven't used the media for Heterobasidion as we tend to use MEA 2% with streptomycin for initial isolation and have reasonable success. My comments may be misguided as I have never used PCNB media and they may be a reason to do this prior to autoclaving but it would be worth clarifying this step." Separate comment: As far as I am aware it is deactivated by autoclaving and autoclaving is the recommended route of disposal of unused Streptomycin solutions to prevent antibiotic resistance. However, the media mentioned in the IPPC protocol is also in the published EPPO protocol PM7/140(1) Heterobasidion irregulare. I have checked the EPPO report from the meeting where the Heterobasidion irregulare protocol was discussed and the issue of autoclaving Streptomycin was not raised other than the recipe instructions were rewritten to make it clearer as to what was added before autoclaving.
G	(General Comment)	C	*Category : TECHNICAL* **(168) EPPO (29 Apr 2024 5:57 PM)** In general, the draft looks fine, but some aspects are missing and sometimes the text is unbalanced with respect to which details

			are covered and which are missing. For example the draft is extremely accurate when it comes to primer concentrations etc. -which of course depends on the specific polymerase used- but does not specify the length of the fragments to be amplified in the reactions, which is considered to be central for a diagnostic standard. This comment relate also to some elements regarding the background and other protocols.
G	(General Comment)	C	*Category : EDITORIAL* **(167) EPPO (29 Apr 2024 5:57 PM)** Only for information to EPPO: Please note that all comments inserted in this Draft text by the Swedish NPPO were kindly provided by researchers and experts on Heterobasidion at the Department of Forest Mycology and Plant Pathology at the Swedish University of Agricultural Sciences (SLU)
G	(General Comment)	C	*Category : TECHNICAL* **(53) Canada (19 Apr 2024 4:27 PM)** Overall, this is a very nice summary with in-depth and thorough coverage on the DP for Heterobasidion annosum sensu lato. The DP listed mainly cover for the detection and identification of five phytopathogenic species with severe economic and environmental losses, including H. abietinum, H. annosum s.s., H. irregulare, H. occidentale and H. parviporum. It is suggested to include a table in either Pest Information or Taxonomic Information to list all authentic phytopathogenic species (H. ecrustosum, H. araucariae, H. amyloideum, H. armandii, H. Australe, H. insulare, H. linzhiense, H. subinsulare, H. subparviporum, H. tibeticum, H. arbitrarium, H. pahangense, H. rutilantiforme) of the genus Heterobasidion, preferably with detailed host range and geographic distributions. According to the description, the LAMP assay serves as a rapid detection protocol with potential for on-site application. It is

			suggested to incorporate a colorimetric reaction to determine the negative and positive as described by Zhou, H.M Yu, J., Liu, Y., Yuan, Y., Wu, C.P., Dai, Y. C., and Chen, J. J. 2023 Rapid detection of Heterobasidion annosum using a loop-mediated isothermal amplification assay. Front Cell Infect Microbiol. 2023 doi: 10.3389/fcimb.2023.1134921. PMID: 37187469; PMCID: PMC10175688. It is recommended that the authors to consider adding another method as a detection and identification protocol using next generation sequencing (NGS) as described by the following reference. Bérubé, J. A., Gagné, P. N., Ponchart, J. P., Phelan, J., Varga, A., & James, D. (2019). Heterobasidion species detected using High Throughput Sequencing (HTS) methods on British Columbia nursery plants. Canadian Journal of Plant Pathology, 41(4), 560–565. https://doi.org/10.1080/07060661.2019.1611665 Finally, detailed information on the components of reaction master mix or taq polymerase is missing and this information is important when repeating an experiment or validating it with another, taking into consideration of any changes may affect the reaction, value and temperature condition. The document has useful information on the biology of the organism. Some references about the metagenomics and some pre-screening HTS work for detection of the pathogen can be included. They will be provided in the text as specific comments.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(26) Guyana (3 Apr 2024 9:32 PM)** Guyana commends the efforts of the IPPC and TP in providing such a relevant and informative protocol. There are no objections at this time
G	(General Comment)	C	*Category : TECHNICAL*

			(1) Congo (1 Feb 2024 5:54 AM) i approve the draft annex to ISPM 27
15	2021-08 ~~Subject proposed during~~ Sujet proposé lors de l'appel à sujets 2021 ~~IPPC call for topics~~de la CIPV.	P	*Category : EDITORIAL* **(56) Mali (28 Apr 2024 1:50 PM)** RAS
43	*Heterobasidion annosum sensu lato* (*s.l.*) (Fr.) Bref., 1888 is a complex of species (also referred to as the *H. annosum* complex) that includes the most common and destructive fungal pathogens of conifers causing root and butt rots. They are widely distributed in the coniferous forests of the northern hemisphere (Korhonen *et al*., 1998; Dai *et al*., 2003; Worrall *et al*., 2010). *H. annosum s.l.* is a major concern especially in intensively managed forests, where the decrease in wood production and the reduction in wood quality caused by the pathogens can result in high economic losses.	C	*Category : EDITORIAL* **(58) EPPO (29 Apr 2024 5:57 PM)** ´s.l.´ in the scientific name should not be in italics, This change should be applied throughout the whole draft.
43	*Heterobasidion annosum sensu lato* (*s.l.*) (Fr.) Bref., 1888 is a complex of species (also referred to as the *H. annosum* complex) that includes the most common and destructive fungal pathogens of conifers causing root and butt rots. They are widely distributed in the coniferous forests of the northern hemisphere (Korhonen *et al*., 1998; Dai *et al*., 2003; Worrall *et al*., 2010). ~~*H.*~~ *Heterobasidion annosum s.l.* is a major concern especially in intensively managed forests, where the decrease in wood production and the reduction in wood quality caused by the pathogens can result in high economic losses.	P	*Category : EDITORIAL* **(57) EPPO (29 Apr 2024 5:57 PM)** It is advised not to start a new sentence with an abbreviation. Pls check throughout the complete document
44	The fungus had long been regarded as a single species until mating experiments revealed the occurrence of intersterile groups within *H. annosum s.l.* (Korhonen, 1978; Chase and Ullrich, 1988; Capretti *et al.*, 1990). A formal description was published for each intersterile group, with the species being named according to their host preferences. Five phytopathogenic species are therefore currently included within *H. annosum s.l.*: *H. abietinum* Niemelä & Korhonen, 1998, *H. annosum* (Fr.) Bref., 1888 *sensu stricto* (*s.s.*), *H. irregulare* Garbel. & Otrosina, 2010, *H. occidentale* Otrosina & Garbel., 2010 and *H. parviporum* Niemelä & Korhonen, 1998. They are characterized based on their geographical origin, host preference, morphology, and biochemical, phylogenetic and genomic traits (Otrosina *et al*., 1993; Linzer *et al*., 2008; Dalman, Olson and Stenlid, 2010; Garbelotto and Gonthier, 2013; Chen *et al*., 2015; Sillo *et al*., 2015; Yuan *et al*., 2021). An additional species, *H. subparviporum* Y.C. Dai, Jia J. Chen & Yuan Yuan, 2021, seems to be a saprotroph (Yuan *et al*., 2021).	C	*Category : EDITORIAL* **(60) EPPO (29 Apr 2024 5:57 PM)** ´s.s.´ in the scientific name should not be in italics, This change should be applied throughout the whole draft.

44	The fungus had long been regarded as a single species until mating experiments revealed the occurrence of intersterile groups within *H. annosum s.l.* (Korhonen, 1978; Chase and Ullrich, 1988; Capretti *et al.*, 1990). A formal description was published for each intersterile group, with the species being named according to their host preferences. Five phytopathogenic species are therefore currently included within *H. annosum s.l.*: *H. abietinum* Niemelä & Korhonen, 1998, *H. annosum* (Fr.) Bref., 1888 *sensu stricto* (*s.s.*), *H. irregulare* Garbel. & Otrosina, 2010, *H. occidentale* Otrosina & Garbel., 2010 and *H. parviporum* Niemelä & Korhonen, 1998. They are characterized based on their geographical ~~origin~~distribution, host preference, morphology, and biochemical, phylogenetic and genomic traits (Otrosina *et al*., 1993; Linzer *et al*., 2008; Dalman, Olson and Stenlid, 2010; Garbelotto and Gonthier, 2013; Chen *et al*., 2015; Sillo *et al*., 2015; Yuan *et al*., 2021). An additional species, *H. subparviporum* Y.C. Dai, Jia J. Chen & Yuan Yuan, 2021, seems to be a saprotroph (Yuan *et al*., 2021).	P	*Category : TECHNICAL* **(59) EPPO (29 Apr 2024 5:57 PM)** also the next as well as the paragraph after that relate to the distribution
45	Three of the phytopathogenic species – *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Niemelä and Korhonen, 1998) – occur in some countries in Europe and Asia, mainly associated with *Abies alba*, *Pinus* spp., and *Picea abies*, respectively (Garbelotto and Gonthier, ~~2013)~~2013) but also found on most *Picea* and *Abies* species and on other conifers e.g. *Pseudotsuga* spp. and *Larix* spp. Depending on tree species composition and structure, these three species of *Heterobasidion* can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: *H. occidentale* (Otrosina and Garbelotto, 2010), associated with *Picea* spp., *Tsuga* spp., *Pseudotsuga* spp. and *Sequoiadendron* spp., and *H. irregulare*, mainly associated with *Pinus* spp., *Juniperus* spp., *Calocedrus decurrens* and *Abies* spp. A table describing the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).	P	*Category : TECHNICAL* **(62) EPPO (29 Apr 2024 5:57 PM)**
45	Three of the phytopathogenic species – *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Niemelä and Korhonen, 1998) – occur in some countries in Europe and Asia, mainly associated with *Abies alba*, *Pinus* spp., and *Picea abies*, respectively (Garbelotto and Gonthier, 2013). Depending on tree species composition and structure, these three species of *Heterobasidion* can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: *H. occidentale* (Otrosina and Garbelotto, 2010), associated	C	*Category : TECHNICAL* **(61) EPPO (29 Apr 2024 5:57 PM)** Occur naturally in North America, but H. irregulare has been introduced to Italy long time ago …

	with *Picea* spp., *Tsuga* spp., *Pseudotsuga* spp. and *Sequoiadendron* spp., and *H. irregulare*, mainly associated with *Pinus* spp., *Juniperus* spp., *Calocedrus decurrens* and *Abies* spp. A table describing the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).		
45	Three of the phytopathogenic species – *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Niemelä and Korhonen, 1998) – ~~occur~~ are found in some countries in Europe and Asia, mainly associated with *Abies alba*, *Pinus* spp., and *Picea abies*, respectively (Garbelotto and Gonthier, 2013). Depending on tree species composition and structure, these three species of *Heterobasidion* can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: *H. occidentale* (Otrosina and Garbelotto, 2010), associated with *Picea* spp., *Tsuga* spp., *Pseudotsuga* spp. and *Sequoiadendron* spp., and *H. irregulare*, mainly associated with *Pinus* spp., *Juniperus* spp., *Calocedrus decurrens* and *Abies* spp.In the article by Garbelotto and Gonthier (2013), it describes outlining the susceptibility of some tree species to H. annosum s.l. species and the main host tissue colonized. ~~A table describing the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).~~	P	*Category : SUBSTANTIVE* **(17) CA (20 Mar 2024 2:31 PM)** (1) In this case, referring to a table without providing its specific content risks generating confusion in the reader. Therefore, it is more appropriate to describe the information directly in the text to ensure that the message is clear and understandable for all readers, without relying on external resources. (2) It is convenient to change the paragraph as suggested to avoid possible confusions in translation and enhance the clarity of the text in English. By using terms like 'are found' or 'are present' instead of 'occur', the presence or existence of phytopathogenic species in certain countries is more accurately conveyed, ensuring that the message is correctly understood by English-speaking readers without leaving room for ambiguous interpretations.
45	Three of the phytopathogenic species – *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Niemelä and Korhonen, 1998) – occur in some countries in Europe and Asia, mainly associated with *Abies alba*, *Pinus* spp., and *Picea abies*, respectively (Garbelotto and Gonthier, 2013). Depending on tree species composition and structure, these three species of *Heterobasidion* can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: *H. occidentale* (Otrosina and Garbelotto, 2010), associated with *Picea* spp., *Tsuga* spp., *Pseudotsuga* spp. and *Sequoiadendron* spp., and *H. irregulare*, mainly associated with *Pinus* spp., *Juniperus* spp., *Calocedrus decurrens* and *Abies* spp. A table describing the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).	C	*Category : EDITORIAL* **(16) CA (20 Mar 2024 2:28 PM)** Three of the phytopathogenic species – H. abietinum, H. annosum s.s. and H. parviporum (Niemelä and Korhonen, 1998) – occur are found in some countries in Europe and Asia, mainly associated with Abies alba, Pinus spp., and Picea abies, respectively (Garbelotto and Gonthier, 2013). Depending on tree species composition and structure, these three species of Heterobasidion can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: H. occidentale (Otrosina and Garbelotto, 2010), associated with Picea spp., Tsuga spp., Pseudotsuga spp. and

			Sequoiadendron spp., and H. irregulare, mainly associated with Pinus spp., Juniperus spp., Calocedrus decurrens and Abies spp. In the article by Garbelotto and Gonthier (2013), it describes outlining the susceptibility of some tree species to H. annosum s.l. species and the main host tissue colonized.A table describing the susceptibility of some tree species to H. annosum s.l. species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).
45	Three of the phytopathogenic species – *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Niemelä and Korhonen, 1998) – ~~occur~~ are found in some countries in Europe and Asia, mainly associated with *Abies alba*, *Pinus* spp., and *Picea abies*, respectively (Garbelotto and Gonthier, 2013). Depending on tree species composition and structure, these three species of *Heterobasidion* can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: *H. occidentale* (Otrosina and Garbelotto, 2010), associated with *Picea* spp., *Tsuga* spp., *Pseudotsuga* spp. and *Sequoiadendron* spp., and *H. irregulare*, mainly associated with *Pinus* spp., *Juniperus* spp., *Calocedrus decurrens* and *Abies* spp. A table describing the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).	P	*Category : EDITORIAL* **(3) Colombia (20 Feb 2024 10:01 PM)** It is convenient to change the paragraph as suggested to avoid possible confusions in translation and enhance the clarity of the text in English. By using terms like 'are found' or 'are present' instead of 'occur', the presence or existence of phytopathogenic species in certain countries is more accurately conveyed, ensuring that the message is correctly understood by English-speaking readers without leaving room for ambiguous interpretations.
45	Three of the phytopathogenic species – *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Niemelä and Korhonen, 1998) – occur in some countries in Europe and Asia, mainly associated with *Abies alba*, *Pinus* spp., and *Picea abies*, respectively (Garbelotto and Gonthier, 2013). Depending on tree species composition and structure, these three species of *Heterobasidion* can coexist in the same conifer stand (e.g. Gonthier, Garbelotto and Nicolotti, 2003). The remaining two species occur in North America: *H. occidentale* (Otrosina and Garbelotto, 2010), associated with *Picea* spp., *Tsuga* spp., *Pseudotsuga* spp. and *Sequoiadendron* spp., and *H. irregulare*, mainly associated with *Pinus* spp., *Juniperus* spp., *Calocedrus decurrens* and *Abies* spp. In the article by Garbelotto and Gonthier (2013), it describes outlining the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized.~~A table describing the susceptibility of some tree species to *H. annosum s.l.* species and the main host tissue colonized is available in Garbelotto and Gonthier (2013).~~	P	*Category : EDITORIAL* **(2) Colombia (20 Feb 2024 10:00 PM)** In this case, referring to a table without providing its specific content risks generating confusion in the reader. Therefore, it is more appropriate to describe the information directly in the text to ensure that the message is clear and understandable for all readers, without relying on external resources.

46	The presence of *H. irregular* in EuropeOne exception to the geographical distribution described above is a relatively recently discovered example of a biological invasion that occurred after the current distribution accidental introduction of this North American species into central Italy during the Second World War (Gonthier *H. irregulare* also includes approximately 100 km of land along the Tyrrhenian coast around Rome (Italy). This is a relatively recently discovered example of a biological invasion that occurred after the accidental introduction of this North American species into central Italy during the Second World War (Gonthier *et al*., 2004). *H. irregulare* is now associated with significant mortality of *Pinus pinea*, in monocultural pine stands, in mixed oak–pine natural forests and in urban parks (Gonthier *et al*., 2007, 2014). In addition, it has been recently reported that *H. irregulare* is replacing *H. annosum* in Tyrrhenian coast (Garbelotto *et al*., 2022), threatening therefore some parts of Europe. In September 2015, *H. irregulare* was included in the A2 list of pests recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO). More information about *H. irregulare* is available in the EPPO Global Database (EPPO, n.d.(a)).	P	*Category : EDITORIAL* **(64) EPPO (29 Apr 2024 5:57 PM)**
46	One exception to the geographical distribution described above is that the current distribution of *H. irregulare* also includes approximately 100 km of land along the Tyrrhenian coast around Rome (Italy). This is a relatively recently discovered example of a biological invasion that occurred after the accidental introduction of this North American species into central Italy during the Second World War (Gonthier *et al*., 2004). *H. irregulare* is now associated with significant mortality of *Pinus pinea*, in monocultural pine stands, in mixed oak–pine natural forests and in urban parks (Gonthier *et al*., 2007, 2014). In September 2015, *H. irregulare* was included in the A2 list of pests recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO). More information about *H. irregulare* is available in the EPPO Global Database (EPPO, n.d.(a)).	C	*Category : EDITORIAL* **(63) EPPO (29 Apr 2024 5:57 PM)** That is a bit of a strange phrasing for us. Why not mentioning „natural distribution" above and speak here of introduction?
47	Although *Heterobasidion* species are characterized by partial interfertility in laboratory experiments, hybrids between the two sympatric North American species, *H. irregulare* and *H. occidentale*, have rarely been found in North America (Garbelotto *et al*., 1996; Lockman *et al*., 2014). Nevertheless, high hybridization rates have been observed between the two naturally allopatric species, *H. annosum s.s.* and *H. irregulare*, in Italian coastal pine woodlands (Gonthier *et al*., 2007).	C	*Category : TECHNICAL* **(66) EPPO (29 Apr 2024 5:57 PM)** Question: Any evidence of hybrids being more aggressive or causing more damage?

47	Although *H. annosum* s.l.~~*Heterobasidion*~~ species are characterized by partial interfertility in laboratory experiments, hybrids between the two sympatric North American species, *H. irregulare* and *H. occidentale*, have rarely been found in North America (Garbelotto *et al*., 1996; Lockman *et al*., 2014). Nevertheless, high hybridization rates have been observed between the two naturally allopatric species, *H. annosum s.s.* and *H. irregulare*, in Italian coastal pine woodlands (Gonthier *et al*., 2007).	P	*Category : EDITORIAL* **(65) EPPO (29 Apr 2024 5:57 PM)**
48	Species of *H. annosum s.l.* have a varied infection biology (Rishbeth, 1950, 1951a, 1951b). Airborne propagules ~~(mostly~~ (almost exclusively basidiospores) infect freshly exposed wood surfaces, such as stump tops or stem and root wounds (primary infection). Once established, the fungus may spread vegetatively via root contacts and grafts (secondary infection) (Stenlid and Redfern, 1998).	P	*Category : EDITORIAL* **(67) EPPO (29 Apr 2024 5:57 PM)**
49	*H. annosum s.l.* commonly develops fruiting bodies producing basidiospores at ground level, at the base of stumps or dead trees, on the roots of fallen trees, on decayed logs left in the forest, or inside the internal cavities of old decayed stumps. *H. annosum s.l.* also produces conidia developing from conidiophores; however, they play an active role only in short-distance dispersal of the fungus (Redfern and Stenlid, 1998) or when accidentally carried by root-feeding insects (Kadlec, Starý and Zumr, 1992). The asexual stage (also referred to as the anamorph) of *H. annosum s.l.* was formerly referred to as *Spiniger meineckellus*.	P	*Category : TECHNICAL* **(69) EPPO (29 Apr 2024 5:57 PM)**
49	*H. annosum s.l.* commonly develops fruiting bodies producing basidiospores at ground level, at the base of stumps or dead trees, or inside the internal cavities of old decayed stumps. *H. annosum s.l.* also produces conidia developing from conidiophores; however, they play an active role only in short-distance dispersal of the fungus (Redfern and Stenlid, 1998) or when accidentally carried by root-feeding insects (Kadlec, Starý and Zumr, 1992). The asexual stage (also referred to as the anamorph) of *H. annosum s.l.* was formerly referred to as *Spiniger meineckellus*.	C	*Category : EDITORIAL* **(68) EPPO (29 Apr 2024 5:57 PM)** The dual nomenclature in mycology is discontinued since 2011, and any reference to the former anamorph name should be avoided. Beside, this one is not very famous, and would not be of any help here.
52	The species complex *H. annosum s.l.* comprises five phytopathogenic species (Chen *et al*., 2015; Yuan *et al*., 2021) responsible for severe economic and environmental losses (Garbelotto and Gonthier, 2013): *H. abietinum*, *H. annosum s.s.*, *H. irregulare*, *H. occidentale* and *H. parviporum*. A sixth species in the complex, *H. subparviporum*, seems to be a saprotroph (Yuan *et al*., 2021).	P	*Category : SUBSTANTIVE* **(71) EPPO (29 Apr 2024 5:57 PM)** H. subparviporum is also a species in the complex. Some rephrasing proposed to keep also the text in line with the text under 1. Pest information also mentioning H. subparviporum as an additional species.
52	The species complex *H. annosum s.l.* comprises five phytopathogenic species (Chen *et al*., 2015; Yuan *et al*., 2021) responsible for severe economic and	C	*Category : SUBSTANTIVE* **(70) EPPO (29 Apr 2024 5:57 PM)**

	environmental losses (Garbelotto and Gonthier, 2013): *H. abietinum*, *H. annosum s.s.*, *H. irregulare*, *H. occidentale* and *H. parviporum*. *H. subparviporum* seems to be a saprotroph (Yuan *et al*., 2021).		This is not fully logical to us. In first sentence, it is stated that the H. Annosum complex comprises 5 species … and at the end of the second paragraph it is stated that „ the protocol refers exclusively to phytopathogenic species of the H. Annosum complex, indicating H. Subparviproum belongs to the complex. It lacks precision here in our eyes. In our opinion, we must decide if the complex is made of 5 or 6 species including H. subparviporum.
62	The detection of *H. annosum s.l.* species in the field is based on the examination of symptoms and the finding of fruiting bodies (Greig, 1998; Gonthier and Thor, 2013). Detection should be complemented by the collection of samples in the field and their subsequent analysis with molecular methods. Molecular methods provide the most reliable way to accurately identify different species, and the only way of distinguishing closely related species of the pathogen. Detection of these pathogens can be achieved by biological (isolation and culture) and molecular methods.	C	*Category : TECHNICAL* **(74) EPPO (29 Apr 2024 5:57 PM)** clarify if the detection is achieved either directly by molecular methods or isolation/culturing followed by molecular methods? Is there an option just to use isolation and culturing for pathogen detection, without molecular confirmation?
62	The detection of *H. annosum s.l.* species in the field is based on the examination of symptoms and the finding of fruiting bodies (Greig, 1998; Gonthier and Thor, 2013). Detection should be complemented by the collection of samples in the field and their subsequent analysis with molecular methods. Molecular methods provide the most reliable way to accurately identify different species, and the ~~only~~ best way of distinguishing closely related species of the pathogen. Detection of these pathogens can be achieved by biological (isolation and culture) and molecular methods.	P	*Category : TECHNICAL* **(73) EPPO (29 Apr 2024 5:57 PM)** other ways possible but this is the best alternative
62	The detection of *H. annosum s.l.* species in the field is based on the examination of symptoms and the finding of fruiting bodies (Greig, 1998; Gonthier and Thor, 2013). Detection should be complemented by the collection of samples in the field and their subsequent analysis with molecular methods. Molecular methods provide the most reliable way to accurately identify different species, and the only way of distinguishing closely related species of the pathogen. Detection of these pathogens can be achieved by biological (isolation and culture) and molecular methods.	C	*Category : TECHNICAL* **(72) EPPO (29 Apr 2024 5:57 PM)** Before only molecular methods are mentioned … incubation for anamorphic production and / or isolation are good ways too … However species distinction is mainly reliable using molecular techniques at the end.
63	This diagnostic protocol describes well-established methods for the detection and identification of different species within the complex *H. annosum s.l.* It is not a comprehensive review of all methods available for the diagnosis of the species within *H. annosum s.l.*	P	*Category : EDITORIAL* **(76) EPPO (29 Apr 2024 5:57 PM)**
63	This diagnostic protocol describes well-established methods for the detection and	C	*Category : TECHNICAL*

	identification of different species within the complex *H. annosum s.l*. It is not a comprehensive review of all methods available for the diagnosis of *H. annosum s.l*.		**(75) EPPO (29 Apr 2024 5:57 PM)** With the exception of H. subparviporum, right?
65	**3.1 Symptoms**	C	*Category : TECHNICAL* **(77) EPPO (29 Apr 2024 5:57 PM)** We suggest to include under this heading or as a separate subsection some information about the risk for wind thrown trees as a consequence of Heterobasidion infections. Heterobasidion parviporum infection in Norway spruce is often associated with wind thrown trees and mortality centers. Mortality centers can also be observed in P. sylvestris stands infected by H. annosum s.s.
66	Two different kinds of symptoms may be ~~observed~~observed inside infected trees, depending on tree species and age: root rot and ~~heart~~ stem rot. Stumps and dead trees should also be examined for the presence of fruiting bodies. Symptoms and fruiting bodies are described below and are illustrated in Figure 1 to Figure 6.	P	*Category : TECHNICAL* **(78) EPPO (29 Apr 2024 5:57 PM)**
68	Tree species characterized by a resinous heartwood (e.g. *Pinus* spp., *Juniperus* spp.) are susceptible to a white root rot and may be killed (Figure 1). In young trees, mortality occurs within a short period of time (i.e. one season); in older trees, mortality proceeds more slowly. Disease symptoms may include the yellowing or browning of foliage, the decline of annual shoot growth, the shedding of old needles (also called the “lion-tailing phenomenon”; Figure 2) and crown transparency. Up to two-thirds of a root system may be killed by the pathogen before symptoms appear in the crown (Greig, 1998).	C	*Category : TECHNICAL* **(79) EPPO (29 Apr 2024 5:57 PM)** question: Is word “white” referring to the paper white mycelium that develops beneath bark? Is this terms generally used in the literature for root rot? If not, avoid using it here. add an explanation if in those plants (resinous heartwood), the infection occurs only in the outer part of the tree (underneath the bark) and does not progress to the heart? In contrast to the heart rot?
69	Heterobasidion symptoms may not be easily distinguished from those of other root-rot agents such as *Armillaria* spp. Pine-infecting *H. annosum s.l*. produces sheets of ~~a~~ white, paper-thin mycelium beneath the bark of infected roots or at the tree collar (Greig, 1998). Although this cannot be regarded as a reliable diagnostic feature of the disease, it is generally sufficient to discriminate between Heterobasidion root rots and Armillaria root rots, whose signs consist of a thicker mycelium or the presence of rhizomorphs (Figure 3; Guillaumin and Legrand, 2013).	P	*Category : EDITORIAL* **(181) New Zealand (3 May 2024 5:47 AM)**
69	Heterobasidion symptoms may not be easily distinguished from those of other root-	C	*Category : EDITORIAL*

	rot agents such as *Armillaria* spp. Pine-infecting *H. annosum s.l.* produces sheets of a white, paper-thin mycelium beneath the bark of infected roots or at the tree collar (Greig, 1998). Although this cannot be regarded as a reliable diagnostic feature of the disease, it is generally sufficient to discriminate between Heterobasidion root rots and Armillaria root rots, whose signs consist of a thicker mycelium or the presence of rhizomorphs (Figure 3; Guillaumin and Legrand, 2013).		**(81) EPPO (29 Apr 2024 5:57 PM)** Is Pine-infecting H.◦annosum◦s.l. referring to H. irregulare and H. annosum s.s ? if so please specify, rather than referring to H. annosum s.l.
69	Symptoms of *Heterobasidion* s.l. infection may not be easily distinguished from those of other root-rot agents such as ~~Heterobasidion symptoms may not be easily distinguished from those of other root-rot agents such as~~ *Armillaria* spp. Pine-infecting *H. annosum s.l.* produces sheets of a white, paper-thin mycelium beneath the bark of infected roots or at the tree collar (Greig, 1998). Although this cannot be regarded as a reliable diagnostic feature of the disease, it is generally sufficient to discriminate between Heterobasidion root rots and Armillaria root rots, whose signs consist of a thicker mycelium or the presence of rhizomorphs (Figure 3; Guillaumin and Legrand, 2013).	P	*Category : EDITORIAL* **(80) EPPO (29 Apr 2024 5:57 PM)**
70	**3.1.2 Heart rot**	C	*Category : TECHNICAL* **(84) EPPO (29 Apr 2024 5:57 PM)** or stem and root rot
70	**3.1.2 ~~Heart~~ Stem rot**	P	*Category : TECHNICAL* **(83) EPPO (29 Apr 2024 5:57 PM)**
70	**3.1.2 Heart rot**	C	*Category : TECHNICAL* **(82) EPPO (29 Apr 2024 5:57 PM)** For those plant species, does infection affect only heart and won't be visible underneath the bark as for root rot ? Or does infection occurs in both places, beneath the bark and heart? Some clarification would be useful.
71	Species characterized by a non-resinous heartwood (e.g. *Picea* spp., *Abies* spp.) are susceptible to an extensive heart rot in the roots, the butt (bole) and the stem (Figure 4). Often the decay is confined to the butt. However, in *Picea abies*, decay columns may extend several metres into the stem. ~~When~~ Even when heart rot develops, in some cases resulting in cavities (Figure 4), external symptoms are rarely visible and mortality does not occur. However, symptoms may include resin flow, bulging of the lower stem and crown deterioration. Mortality has been observed in saplings and in very young trees. Foliage yellowing can be observed in cases of advanced decay (Greig, 1998).	P	*Category : EDITORIAL* **(85) EPPO (29 Apr 2024 5:57 PM)**
72	In *Picea abies*, *Armillaria borealis* Marxm. & Korhonen, 1982 causes a dark	C	*Category : TECHNICAL*

	brown rot leading to hollowing of the tree earlier than if infected by *H. parviporum*. However, in contrast to *Armillaria* spp., the Heterobasidion decayed wood has a characteristic appearance of “pocket rot” and stays rather light in colour for a long time, and fresh heart rot is often surrounded by a violet-coloured ring.		**(86) EPPO (29 Apr 2024 5:57 PM)** Even if Heterobasidion decay initially has a red-brownish colour, the terms red rot and brown rot should not be used. Describe the rot in a different manner e.g. ‘a darker coloured decay’ or ‘a dark, wet rot’. And why only mention A. borealis, when A. ostoya is much more common and aggressive on conifers ?
72	In *Picea abies*, *Armillaria borealis* Marxm. & Korhonen, 1982 causes a dark brown rot leading to hollowing of the tree earlier than if infected by *H. parviporum*. However, in contrast to *Armillaria* spp., the Heterobasidion decayed wood has a characteristic appearance of “pocket rot” and stays rather light in colour for a long time, and fresh heart rot is often surrounded by a violet-coloured ring.	C	*Category : EDITORIAL* **(18) CA (20 Mar 2024 2:33 PM)** It would be appropriate to include a figure illustrating "pocket rot" within the same paragraph where this condition is described. This would provide a more comprehensive and visual understanding of the phenomenon without the reader having to resort to external sources. This would help strengthen the writing by providing a direct visual reference that complements the textual description of the phenomenon.
72	In *Picea abies*, *Armillaria borealis* Marxm. & Korhonen, 1982 causes a dark brown rot leading to hollowing of the tree earlier than if infected by *H. parviporum*. However, in contrast to *Armillaria* spp., the Heterobasidion decayed wood has a characteristic appearance of “pocket rot” and stays rather light in colour for a long time, and fresh heart rot is often surrounded by a violet-coloured ring.	C	*Category : TECHNICAL* **(4) Colombia (20 Feb 2024 10:14 PM)** It would be appropriate to include a figure illustrating "pocket rot" within the same paragraph where this condition is described. This would provide a more comprehensive and visual understanding of the phenomenon without the reader having to resort to external sources. This would help strengthen the writing by providing a direct visual reference that complements the textual description of the phenomenon.
74	Fruiting bodies of *H. annosum s.l.* most commonly develop at ground level, at the base of stumps or dead trees, or inside the internal cavities of old decayed stumps; they may envelop litter debris (Figure 5). Fruiting bodies may be produced more intensively in the rainy and humid periods of the year. Unfortunately, identifications in the field may be erroneous because morphological traits partially overlap between species and small differences occur. In general, fruiting bodies are perennial, pileate, resupinate or effused-reflexed (partly pileate, partly resupinate), rubbery in texture, and 1–40 cm across. The top surface is reddish or dark brown in colour (Figure 6) and becomes darker with age. The margin is distinct and white. The lower surface is white or cream coloured and is characterized by numerous	P	*Category : TECHNICAL* **(88) EPPO (29 Apr 2024 5:57 PM)**

	small pores. Incipient fruiting bodies may form ~~under~~ outside the bark. These are smaller in size (0.5–1 cm in diameter) and are referred to as "popcorn" because of their appearance (Otrosina and Garbelotto, 2010; Figure 6).		
74	Fruiting bodies of *H. annosum s.l.* most commonly develop at ground level, at the base of stumps or dead trees, or inside the internal cavities of old decayed stumps; they may envelop litter debris (Figure 5). Fruiting bodies may be produced more intensively in the rainy and humid periods of the year. Unfortunately, identifications in the field may be erroneous because morphological traits partially overlap between species and small differences occur. In general, fruiting bodies are perennial, pileate, resupinate or effused-reflexed (partly pileate, partly resupinate), rubbery in texture, and 1–40 cm across. The top surface is reddish or dark brown in colour (Figure 6) and becomes darker with age. The margin is distinct and white. The lower surface is white or cream coloured and is characterized by numerous small pores. Incipient fruiting bodies may form under the bark. These are smaller in size (0.5–1 cm in diameter) and are referred to as "popcorn" because of their appearance (Otrosina and Garbelotto, 2010; Figure 6).	C	*Category : TECHNICAL* **(87) EPPO (29 Apr 2024 5:57 PM)** We suggest to add to the first sentence: ´on roots of wind thrown trees, especially Norway spruce.´
76	Different techniques for sampling and sample preparation as described below are recommended depending on the material being tested. With the exception of passive spore traps, samples should be kept cool and sent to the laboratory in closed plastic or paper bags or containers for isolation the following day. However, in sealed self-closing plastic bags, excessive moisture can hasten tissue degradation and saprophytic activity. Storage at low temperatures (2–8 °C) is highly recommended to prolong sample life.	C	*Category : TECHNICAL* **(90) EPPO (29 Apr 2024 5:57 PM)** Information is missing that state that there is a risk of missing the infected tissue when sampling wood from symptomatic trees. At least a few sentences about this would be good, either in this section or under 3.2.2. and 3.2.3.
76	Different techniques for sampling and sample preparation as described below are recommended depending on the material being tested. With the exception of passive spore traps, samples should be kept cool and sent to the laboratory in closed plastic or paper bags or containers for isolation and culture at the latest on the following day. However, in sealed self-closing plastic bags, excessive moisture can hasten tissue degradation and saprophytic activity. Storage at low temperatures (2–8 °C) is highly recommended to prolong sample life.	P	*Category : TECHNICAL* **(89) EPPO (29 Apr 2024 5:57 PM)**
81	Wood samples (at least 10 × 10 × 5 mm) from living trees that are symptomatic or suspected of being infected should be collected with a hammer and a chisel from the outer sapwood of roots or the tree collar or from stumps or logs. The chisel must be disinfected with 95% ethanol (v/v) at the beginning of sample taking and after every sampling. Wood samples should be placed in a sealed container or in	P	*Category : EDITORIAL* **(92) EPPO (29 Apr 2024 5:57 PM)**

	closed plastic or paper bags and stored at 2–8 °C until laboratory analyses. Subsequently, they should be incubated at room temperature (18–24 °C) under moist conditions for 7–15 days to promote development of mycelium and conidia (anamorph stage). ~~Wood samples (at least 10 × 10 × 5 mm) from living trees that are symptomatic or suspected of being infected should be collected with a hammer and a chisel from the outer sapwood of roots or the tree collar or from stumps or logs. The chisel must be disinfected with 95% ethanol (v⁄v) after every sampling. Wood samples should be placed in a sealed container or in closed plastic or paper bags and stored at 2–8 °C until laboratory analyses. Subsequently, they should be incubated at room temperature (18–24 °C) under moist conditions for 7–15 days.~~		
81	Wood samples (at least 10 × 10 × 5 mm) from living trees that are symptomatic or suspected of being infected should be collected with a hammer and a chisel from the outer sapwood of roots or the tree collar or from stumps or logs. The chisel must be disinfected with 95% ethanol (v⁄v) after every sampling. Wood samples should be placed in a sealed container or in closed plastic or paper bags and stored at 2–8 °C until laboratory analyses. Subsequently, they should be incubated at room temperature (18–24 °C) under moist conditions for 7–15 days.	C	*Category : TECHNICAL* **(91) EPPO (29 Apr 2024 5:57 PM)** Normally, we remove first the upper surface of the wood (2-3 mm) to avoid external contamination, then disinfect chisel again and take the sample.
82	Wood samples can be also collected from living trees by extracting wood cores by means of an increment borer. The increment borer must be disinfected with 95% ethanol (v⁄v) after every drilling. Wood cores may be incubated in sterile plastic bottles in the dark at room temperature (18–24 °C) for up to one month.	C	*Category : SUBSTANTIVE* **(94) EPPO (29 Apr 2024 5:57 PM)** Also add the expectation of incubating the wood cores
82	Wood samples can be also collected from living trees by extracting wood cores by means of an increment borer. The increment borer must be disinfected with 95% ethanol (v⁄v) at the beginning of sample takting and after every drilling. Wood cores may be incubated in sterile plastic bottles in the dark at room temperature (18–24 °C) for up to one month.	P	*Category : EDITORIAL* **(93) EPPO (29 Apr 2024 5:57 PM)**
82	Wood samples can be also collected from living trees by extracting wood cores by means of an increment borer. The increment borer must be disinfected with ~~95%~~ 75% ethanol (v⁄v) after every drilling. Wood cores may be incubated in sterile plastic bottles in the dark at room temperature (18–24 °C) for up to one month.	P	*Category : TECHNICAL* **(55) China (24 Apr 2024 7:57 AM)** Here should be 75% not 95%
85	**3.2.3 Wood sawdust from drillings of symptomatic (or suspected) and asymptomatic trees**	C	*Category : TECHNICAL* **(19) CA (20 Mar 2024 2:35 PM)** At the end of the procedure, Do you put sellant in the hole? Please, clarify the question. If possible, include the option

85	**3.2.3 Wood sawdust from drillings of symptomatic (or suspected) and asymptomatic trees**	C	*Category : TECHNICAL* **(14) Colombia (28 Feb 2024 8:09 PM)** At the end of the procedure, Do you put sellant in the hole? Please, clarify the question. If possible, include the option
86	Wood sawdust should be taken from drillings conducted at the base of symptomatic, suspected and asymptomatic trees, with a drill long enough to reach the heartwood in order to obtain wood decayed by *Heterobasidion* sp. (e.g. with a 4-mm-diameter, 43-cm-long drill bit). Four holes at 90° from one another should be drilled at the base of the trunk (approximately 5 cm above ground) (Guglielmo *et al*., 2010). Two samples should be obtained by combining opposite drillings in 9-cm-diameter plastic Petri dishes (Figure 7) or in tubes and used for the molecular detection of the pathogen; alternatively, all the wood sawdust generated (from the four drillings) can be pooled together in one sample (Guglielmo *et al*., 2010). After each sampling, the drill bit must be disinfected with 0.5% (w/v) sodium hypochlorite (NaClO) solution, rinsed with sterile distilled water, and wiped with 70% or 95% ethanol (v/v).	C	*Category : TECHNICAL* **(98) EPPO (29 Apr 2024 5:57 PM)** Why is this different from the disinfection method in 3.2.2?
86	Wood sawdust should be taken from drillings conducted at the base of symptomatic, suspected and asymptomatic trees, with a drill long enough to reach the heartwood in order to obtain wood decayed by *Heterobasidion* sp. (e.g. with a 4-mm-diameter, 43-cm-long drill bit). Four holes at 90° from one another should be drilled at the base of the trunk (approximately 5 cm above ground) (Guglielmo *et al*., 2010). Two samples should be obtained by combining opposite drillings in 9-cm-diameter plastic Petri dishes (Figure 7) or in tubes and used for the molecular detection of the pathogen; alternatively, all the wood sawdust generated (from the four drillings) can be pooled together in one sample (Guglielmo *et al*., 2010). After each sampling, the drill bit must be disinfected with 0.5% (w/v) sodium hypochlorite (NaClO) solution, rinsed with sterile distilled water, and wiped with 70% or 95% ethanol (v/v).	C	*Category : TECHNICAL* **(97) EPPO (29 Apr 2024 5:57 PM)** This section is far more (or unnecessary ?) specific compared to the above section 3.2.2.
86	Wood sawdust should be taken from drillings conducted at the base of symptomatic, suspected and asymptomatic trees, with a drill long enough to reach the heartwood in order to obtain wood decayed by *Heterobasidion* sp. (e.g. with a 4-mm-diameter, 43-cm-long drill bit). Four holes at 90° from one another should be drilled at the base of the trunk (approximately 5 cm above ground) (Guglielmo *et al*., 2010). Two samples should be obtained by combining opposite drillings in 9-cm-diameter plastic Petri dishes (Figure 7) or in tubes and used for	C	*Category : TECHNICAL* **(96) EPPO (29 Apr 2024 5:57 PM)** Why are there two sterilization methods one using 95% Ethanol recommended for the chisel and another combining NaClO and ethanol for the drill. What is the rational for that?

	the molecular detection of the pathogen; alternatively, all the wood sawdust generated (from the four drillings) can be pooled together in one sample (Guglielmo *et al*., 2010). After each sampling, the drill bit must be disinfected with 0.5% (w/v) sodium hypochlorite (NaClO) solution, rinsed with sterile distilled water, and wiped with 70% or 95% ethanol (v/v).		
86	Wood sawdust should be taken from drillings conducted at the base of symptomatic, suspected and asymptomatic trees, with a drill long enough to reach the heartwood in order to obtain wood decayed by *Heterobasidion* sp. (e.g. with a 4-mm-diameter, 43-cm-long drill bit). Four holes at 90° from one another should be drilled at the base of the trunk (approximately 5 cm above ground) (Guglielmo *et al*., 2010). Two samples should be obtained by combining opposite drillings in 9-cm-diameter plastic Petri dishes (Figure 7) or in tubes and used for the molecular detection of the pathogen; alternatively, all the wood sawdust generated (from the four drillings) can be pooled together in one sample (Guglielmo *et al*., 2010). After each sampling, the drill bit must be disinfected with 0.5% (w/v) sodium hypochlorite (NaClO) solution, rinsed with sterile distilled water, and wiped with 70% or 95% ethanol (v/v).	C	*Category : TECHNICAL* **(95) EPPO (29 Apr 2024 5:57 PM)** No removing of the bark before drilling? In order to avoid other epiphytic / endophytic fungi.
87	The diagnostic efficiency is higher in smaller trees (diameter at breast height 1.30 m above ground (DBH) <80 cm) than in larger ones (DBH >80 cm) (Guglielmo *et al*., 2010). In the latter case, or in the presence of monumental specimens, it may be useful to increase the number of drillings and to analyse the sawdust from each drilling separately to maximize the diagnostic efficiency. However, the choice of the most appropriate sampling method may vary depending on considerations such as tree diameter, acceptable injury levels, cost and the diagnostic sensitivity that is needed (Guglielmo *et al*., 2010).	C	*Category : SUBSTANTIVE* **(99) EPPO (29 Apr 2024 5:57 PM)** This is also valid for the section 3.2.2., so perhaps some restructuring of text could be considered.
90	**3.2.4 Passive spore traps**	C	*Category : TECHNICAL* **(100) EPPO (29 Apr 2024 5:57 PM)** We suggest not to call this section Passive spore traps. Use ´wood disc for trapping spores or trapping of spores on wood discs´. There exists other devices/equipment to serve as and called passive or active spore traps.
90	**3.2.4 Passive spore traps**	C	*Category : TECHNICAL* **(51) Canada (19 Apr 2024 4:15 PM)** A section on HTS use could be add. Ref. Tremblay ÉD, Duceppe MO, Bérubé JA,

			Kimoto T, Lemieux C, Bilodeau GJ. Screening for Exotic Forest Pathogens to Increase Survey Capacity Using Metagenomics. Phytopathology. 2018 Dec;108(12):1509-1521. doi: 10.1094/PHYTO-02-18-0028-R. Epub 2018 Nov 2. PMID: 29923801. Bérubé, J. A., Gagné, P. N., Ponchart, J. P., Phelan, J., Varga, A., & James, D. (2019). Heterobasidion species detected using High Throughput Sequencing (HTS) methods on British Columbia nursery plants. Canadian Journal of Plant Pathology, 41(4), 560–565. https://doi.org/10.1080/07060661.2019.1611665
91	Spores of *H. annosum s.l.* can be trapped using the modified version of the wood-disc exposure method (Rishbeth, 1959; James and Cobb, 1984; Gonthier *et al*., 2007). Freshly cut wood discs, approximately 11–13 cm in diameter, 0.5–1.5 cm in thickness and without bark, cut the day before their exposure in the forest from living and healthy *Picea abies* or *Pinus* spp., should be sprayed with 65% ethanol (v/v) and exposed for 24 hours in the field. Discs should be placed singly in open, 15-cm diameter, plastic Petri dishes containing sterile pieces of filter paper dampened with 3.5 mL of sterile distilled water (Figure 7) to prevent drying during exposure. Discs in closed Petri dishes should also be included as controls to check for possible contamination with either *H. annosum s.l.* already present in the wood discs used as traps or airborne spores that landed on the wood during trap preparation. After field exposure, the filter papers on which the wood discs are placed should be replaced by new filter papers dampened with sterile distilled water in the laboratory. The wood discs are then incubated at room temperature (18–24 °C) for 7–15 days. The discs should be regularly checked for possible *H. annosum s.l.* colonies after a week in case such colonies subsequently become overgrown by other fungi (e.g. *Ophiostoma* spp., *Penicillium* spp., *Trichoderma* spp.).	C	*Category : TECHNICAL* **(103) EPPO (29 Apr 2024 5:57 PM)** It is mentioned in section 3.3.2 to look under the microscope for typical conidiophores. Any indication how the possible colonies could look with unaided eye?
91	Spores of *H. annosum s.l.* can be trapped using the modified version of the wood-disc exposure method (Rishbeth, 1959; James and Cobb, 1984; Gonthier *et al*., 2007). Freshly cut wood discs, approximately 11–13 cm in diameter, 0.5–1.5 cm in thickness and without bark, cut the day before their exposure in the forest from living and healthy *Picea abies* or *Pinus* spp., should be sprayed with 65% ethanol	P	*Category : EDITORIAL* **(102) EPPO (29 Apr 2024 5:57 PM)** not needed

	(v/v) and exposed for 24 hours in the field. Discs should be placed singly in open, 15-cm diameter, plastic Petri dishes containing sterile pieces of filter paper dampened with 3.5 mL of sterile distilled water (Figure 7) to prevent drying during exposure. Discs in closed Petri dishes should also be included as controls to check for possible contamination with ~~either~~ *H. annosum s.l.* already present in the wood discs used as traps or airborne spores that landed on the wood during trap preparation. After field exposure, the filter papers on which the wood discs are placed should be replaced by new filter papers dampened with sterile distilled water in the laboratory. The wood discs are then incubated at room temperature (18–24 °C) for 7–15 days. The discs should be regularly checked for possible *H. annosum s.l.* colonies after a week in case such colonies subsequently become overgrown by other fungi (e.g. *Ophiostoma* spp., *Penicillium* spp., *Trichoderma* spp.).		
91	Spores of *H. annosum s.l.* can be trapped using the modified version of the wood-disc exposure method (Rishbeth, 1959; James and Cobb, 1984; Gonthier *et al*., 2007). Freshly cut wood discs, approximately 11–13 cm in diameter, 0.5–1.5 cm in thickness and without bark, cut the day before their exposure in the forest from living and healthy *Picea abies* or *Pinus* spp., should be sprayed with 65% ethanol (v/v) and exposed for 24 hours in the field. Discs should be placed singly in open, 15-cm diameter, plastic Petri dishes containing sterile pieces of filter paper dampened with 3.5 mL of sterile distilled water (Figure 7) to prevent drying during exposure. Discs in closed Petri dishes should also be included as controls to check for possible contamination with either *H. annosum s.l.* already present in the wood discs used as traps or airborne spores that landed on the wood during trap preparation. After field exposure, the filter papers on which the wood discs are placed should be replaced by new filter papers dampened with sterile distilled water in the laboratory. The wood discs are then incubated at room temperature (18–24 °C) for 7–15 days. The discs should be regularly checked for possible *H. annosum s.l.* colonies after a week in case such colonies subsequently become overgrown by other fungi (e.g. *Ophiostoma* spp., *Penicillium* spp., *Trichoderma* spp.).	C	*Category : EDITORIAL* **(101) EPPO (29 Apr 2024 5:57 PM)** Colonies = typical mycelium?
94	Isolation of *H. annosum s.l.* may be performed directly from fresh fruiting bodies or from infected wood samples or passive spore traps incubated in moist conditions as described above (see sections 3.2.2 and 3.2.4, respectively). Isolation of *H. annosum s.l.* is not feasible from non-sterile wood sawdust.	P	*Category : TECHNICAL* **(105) EPPO (29 Apr 2024 5:57 PM)**

94	Isolation of *H. annosum s.l.* may be performed directly from fresh fruiting bodies or from infected wood samples or passive spore traps incubated in moist conditions as described above (see sections 3.2.2 and 3.2.4, respectively). Isolation of *H. annosum s.l.* is not feasible from wood sawdust.	C	*Category : TECHNICAL* **(104) EPPO (29 Apr 2024 5:57 PM)** See previous comments on traps
96	Pieces of tissue approximately 2 × 2 × 5 mm in size should be excised using a sterile scalpel from the context of fruiting bodies, surface-disinfected in a 30% (w⁄v) hydrogen peroxide (H_2O_2) solution or in a 5% (w⁄v) sodium hypochlorite (NaOCl) solution for eight seconds and rinsed three times in sterile distilled water for approximately ten seconds (Giordano *et al*., 2009). Then, the pieces of tissue are transferred onto plastic Petri dishes filled with pentachloronitrobenzene (PCNB)-based selective culture medium for *H. annosum s.l.* (Table 1; Kuhlman and Hendrix, 1962) or with the semi-selective culture medium modified from Legrand and Guillaumin (1993) by Bendel *et al*. (2006) (Table 2). Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light.	C	*Category : TECHNICAL* **(109) EPPO (29 Apr 2024 5:57 PM)** This seems an excessive use of chemicals. It is sufficient o spray the surface of the fruit body with 70% ethanol, make surface cuts with a disinfected scalpel a couple of times to expose the inside of the hat flesh (trama) and take out sterile samples.
96	Pieces of tissue approximately 2 × 2 × 5 mm in size should be excised using a sterile scalpel from the context of fruiting bodies, surface-disinfected in a 30% (w⁄v) hydrogen peroxide (H_2O_2) solution or in a 5% (w⁄v) sodium hypochlorite (NaOCl) solution for eight seconds and rinsed three times in sterile distilled water for approximately ten seconds (Giordano *et al*., 2009). Then, they are transferred onto plastic Petri dishes filled with pentachloronitrobenzene (PCNB)-based selective culture medium for *H. annosum s.l.* (Table 1; Kuhlman and Hendrix, 1962) or with the semi-selective culture medium modified from Legrand and Guillaumin (1993) by Bendel *et al*. (2006) (Table 2). Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light.	C	*Category : TECHNICAL* **(108) EPPO (29 Apr 2024 5:57 PM)** Could those alternatively be placed in the incubators with light? I think some of labs avoid culturing on the benches those days.
96	Pieces of tissue approximately 2 × 2 × 5 mm in size should be excised using a sterile scalpel from the context of fruiting bodies, surface-disinfected in a 30% (w⁄v) hydrogen peroxide (H_2O_2) solution or in a 5% (w⁄v) sodium hypochlorite (NaOCl) solution for eight seconds and rinsed three times in sterile distilled water for approximately ten seconds (Giordano *et al*., 2009). Then, they are transferred onto plastic Petri dishes filled with pentachloronitrobenzene (PCNB)-based selective culture medium for *H. annosum s.l.* (Table 1; Kuhlman and Hendrix, 1962) or with the semi-selective culture medium modified from Legrand and Guillaumin (1993) by Bendel *et al*. (2006) (Table 2). Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light.	C	*Category : TECHNICAL* **(107) EPPO (29 Apr 2024 5:57 PM)** Is this standard procedure?
96	Pieces of tissue approximately 2 × 2 × 5 mm in size should be excised using a	P	*Category : EDITORIAL* **(106) EPPO (29 Apr 2024 5:57 PM)**

	sterile scalpel from the context of fruiting bodies, surface-disinfected in a 30% (w/v) hydrogen peroxide (H_2O_2) solution or in a 5% (w/v) sodium hypochlorite (NaOCl) solution for eight seconds and rinsed three times in sterile distilled water for approximately ten seconds (Giordano *et al*., 2009). Then, ~~they~~ the pieces of tissue are transferred onto plastic Petri dishes filled with pentachloronitrobenzene (PCNB)-based selective culture medium for *H. annosum s.l.* (Table 1; Kuhlman and Hendrix, 1962) or with the semi-selective culture medium modified from Legrand and Guillaumin (1993) by Bendel *et al*. (2006) (Table 2). Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light.		
97	Cultures can also be obtained from spore prints. To accomplish this, the lid of a Petri dish is propped at an angle over the Petri dish, with the fruiting body wedged between the lid and the edge of the Petri dish (but without touching the agar medium). This assembly is then incubated until spores are released on the agar surface.	C	*Category : TECHNICAL* **(110) EPPO (29 Apr 2024 5:57 PM)** Should this also include isolation of single spore cultures?
97	Cultures can also be obtained from spore prints. To accomplish this, the lid of a Petri dish is propped at an angle over the Petri dish, with the fruiting body wedged between the lid and the edge of the Petri dish (but without touching the agar medium). This assembly is then incubated until spores are released on the agar surface.	C	*Category : TECHNICAL* **(20) CA (20 Mar 2024 2:36 PM)** The provided description may not be clear enough for the reader, as the manipulation and arrangement of the Petri dish lid can be difficult to visualize without a graphical representation. Therefore, it would be appropriate to complement this explanation with a diagram or figure that clearly illustrates the described process. This would help readers better understand how the procedure is carried out and ensure proper execution when culturing from spore prints.
97	Cultures can also be obtained from spore prints. To accomplish this, the lid of a Petri dish is propped at an angle over the Petri dish, with the fruiting body wedged between the lid and the edge of the Petri dish (but without touching the agar medium). This assembly is then incubated until spores are released on the agar surface.	C	*Category : TECHNICAL* **(5) Colombia (20 Feb 2024 10:18 PM)** The provided description may not be clear enough for the reader, as the manipulation and arrangement of the Petri dish lid can be difficult to visualize without a graphical representation. Therefore, it would be appropriate to complement this explanation with a diagram or figure that clearly illustrates the described process. This would help readers better understand how the procedure is carried out and ensure proper execution when culturing from spore prints.
98	Subcultures can be maintained on generic agar-based media (e.g. malt extract agar,	C	*Category : TECHNICAL* **(111) EPPO (29 Apr 2024 5:57 PM)**

	potato dextrose agar). Despite a lower success rate because of the more abundant presence of contaminant colonies (e.g. *Penicillium* spp., *Trichoderma* spp.), isolation of *H. annosum s.l.* can also be performed on these generic agar-based media.		Should it be added supplemented with antibiotics, e.g streptomycin? Other mentioned media seem to have antibiotics.
114	- Pentachloronitrobenzene (PCNB)	C	*Category : TECHNICAL* **(44) Canada (19 Apr 2024 3:27 PM)** The PCNB needs to be diluted in EtOH, the info should be added or in the note
156	**3.3.2 Isolation from wood samples and passive spore traps**	C	*Category : TECHNICAL* **(112) EPPO (29 Apr 2024 5:57 PM)** See previous comment above
157	For isolation from wood samples and passive spore traps, individual colonies can be identified under a dissecting microscope (65–160× magnification) based on the presence of conidiophores of the anamorphic stage of the fungus. The conidiophores appear as a mass of whitish “pinheads” on stalks (Figure 7). Hyphae and conidiophores should be directly transferred onto plastic Petri dishes filled with either the selective or semi-selective culture medium described above (see section 3.3.1). In the case of passive spore traps, when *Heterobasidion* spp. colonies are present, 3–5 colonies per disc should be isolated. Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light. Subcultures can be maintained on generic agar-based media as described above (see section 3.3.1).	C	*Category : TECHNICAL* **(115) EPPO (29 Apr 2024 5:57 PM)** Possible to use generic agar as mentioned above?
157	For isolation from wood samples and passive spore traps, individual colonies can be identified under a dissecting microscope (65–160× magnification) based on the presence of conidiophores of the anamorphic stage of the fungus. The conidiophores appear as a mass of whitish “pinheads” on stalks (Figure 7). Hyphae and conidiophores should be directly transferred onto plastic Petri dishes filled with either the selective or semi-selective culture medium described above (see section 3.3.1). In the case of passive spore traps, when *Heterobasidion* spp. colonies are present, 3–5 colonies per disc should be isolated. Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light. Subcultures can be maintained on generic agar-based media as described above (see section 3.3.1).	C	*Category : TECHNICAL* **(114) EPPO (29 Apr 2024 5:57 PM)** Is this standard?
157	For isolation from wood samples and passive spore traps, individual colonies can be identified under a dissecting microscope (65–160× magnification) based on the presence of conidiophores of the anamorphic stage of the fungus. The conidiophores appear as a mass of whitish “pinheads” on stalks (Figure 7). Hyphae	C	*Category : TECHNICAL* **(113) EPPO (29 Apr 2024 5:57 PM)** 100?

	and conidiophores should be directly transferred onto plastic Petri dishes filled with either the selective or semi-selective culture medium described above (see section 3.3.1). In the case of passive spore traps, when *Heterobasidion* spp. colonies are present, 3–5 colonies per disc should be isolated. Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light. Subcultures can be maintained on generic agar-based media as described above (see section 3.3.1).		
157	For isolation from wood samples and passive spore traps, individual colonies can be identified under a dissecting microscope (65–160× magnification) based on the presence of conidiophores of the anamorphic stage of the fungus. The conidiophores appear as a mass of whitish "pinheads" on stalks (Figure 7). Hyphae and conidiophores should be directly transferred onto plastic Petri dishes filled with either the selective or semi-selective culture medium described above (see section 3.3.1). In the case of passive spore traps, when *Heterobasidion* spp. colonies are present, 3–5 colonies per disc should be isolated. Petri dishes are incubated at room temperature (18–24 °C) for at least 4–5 days in the light. Subcultures can be maintained on generic agar-based media as described above (see section 3.3.1).	C	*Category : TECHNICAL* **(45) Canada (19 Apr 2024 3:30 PM)** Why does this value matter if different?
159	**3.4 Detection by molecular methods**	C	*Category : SUBSTANTIVE* **(116) EPPO (29 Apr 2024 5:57 PM)** A major improvement of the use of this standard would consist in providing a flow diagram giving the different possibilities of combining different tests to achieve a reliable detection and identification
160	Molecular methods have been developed to detect pathogenic species or groups of species in *H. annosum s.l.* using conventional polymerase chain reaction (PCR), real-time PCR, multiplex real-time PCR or loop-mediated isothermal amplification (LAMP) (Table 3).[1]	C	*Category : TECHNICAL* **(117) EPPO (29 Apr 2024 5:57 PM)** Consider the use of the term quantitative PCR (q-PCR) that is the more commonly used term
162	For this diagnostic protocol, six methods (or sets of methods) have been selected based on laboratories' experience of using them and the availability of validation data: one method for the detection of *H. annosum s.l.* (species complex) and five species-specific methods (or sets of methods) to detect different ~~*Heterobasidion*~~*H. annosum* ~~species. These methods are described below~~s.l. species. These methods are described below.	P	*Category : EDITORIAL* **(119) EPPO (29 Apr 2024 5:57 PM)**
162	For this diagnostic protocol, six methods (or sets of methods) have been selected based on laboratories' experience of using them and the availability of	C	*Category : TECHNICAL* **(118) EPPO (29 Apr 2024 5:57 PM)**

	validation data: one method for the detection of *H. annosum s.l.* (species complex) and five species-specific methods (or sets of methods) to detect different *Heterobasidion* species. These methods are described below.		Does it mean this first method would detect Hsubparviporum too?
162	For this diagnostic protocol, six methods (or sets of methods) have been selected based on laboratories' experience of using them and the availability of validation data: one method for the detection of *H. annosum s.l.* (species complex) and five species-specific methods (or sets of methods) to detect different *Heterobasidion* species. These methods are described below.	C	*Category : TECHNICAL* **(27) United States of America (5 Apr 2024 4:25 PM)** Validation data to support these assay choices is very favorable. Indicates that they have sufficient utility
163	For practical purposes, Bahnweg *et al*. (2002) developed specific PCR primers for *H. annosum s.l.* allowing simultaneous detection of *Armillaria* spp. in multiplex PCR. This method can be applied to detect these two pathogens causing tree root rot, particularly in the early stages of infection in which symptoms are not yet clearly expressed (see sections 3.1.1 and 3.1.2). Both *H. annosum s.l.* and *Armillaria* spp. are economically-important pathogens of conifers that can coexist in the same stand and sometimes double infection is possible. In the absence of signs of these two pathogens (e.g. fruiting bodies, rhizomorphs for *Armillaria* spp.), ~~*Heterobasidion*~~*H. annosum* ~~symptoms may not be easily distinguished from those of~~ s.l. symptoms may not be easily distinguished from those of *Armillaria* spp. or of other root-rot agents.	P	*Category : EDITORIAL* **(121) EPPO (29 Apr 2024 5:57 PM)**
163	For practical purposes, Bahnweg *et al*. (2002) developed specific PCR primers for *H. annosum s.l.* allowing simultaneous detection of *Armillaria* spp. in multiplex PCR. This method can be applied to detect these two pathogens causing tree root rot, particularly in the early stages of infection in which symptoms are not yet clearly expressed (see sections 3.1.1 and 3.1.2). Both *H. annosum s.l.* and *Armillaria* spp. are economically-important pathogens of conifers that can coexist in the same stand and sometimes double infection is possible. In the absence of signs of these two pathogens (e.g. fruiting bodies, rhizomorphs for *Armillaria* spp.), *Heterobasidion* symptoms may not be easily distinguished from those of *Armillaria* spp. or of other root-rot agents.	C	*Category : TECHNICAL* **(120) EPPO (29 Apr 2024 5:57 PM)** Co-infection?
164	Methods based on PCR targeting mitochondrial DNA have been developed to discriminate the species *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Gonthier *et al*., 2001; Gonthier, Garbelotto and Nicolotti, 2003). A duplex PCR-based method targeting nuclear and mitochondrial DNA has also been developed to distinguish between *H. annosum s.s.* and *H. irregulare* (Gonthier *et al*., 2007) and to detect hybrids between these two species. In addition, real-time PCR methods	P	*Category : EDITORIAL* **(122) EPPO (29 Apr 2024 5:57 PM)**

	have been developed. The internal transcribed spacer (ITS) region was selected by Lamarche *et al*. (2017) to design a set of real-time PCR methods using hydrolysis probes with different levels of specificity to detect the species of *H. annosum s.l.* occurring in North America, *H. irregulare* and *H. occidentale*, and *H. annosum s.s.* Ioos *et al*. (2019) developed multiplex real-time PCR methods for the detection and identification of ~~*Heterobasidion*~~*H. annosum* ~~species infecting conifers in Europe~~ s.l. species infecting conifers in Europe (*H. abietinum*, *H. annosum s.s.*, *H. irregulare* and *H. parviporum*), which can be used simultaneously or individually thanks to probes labelled with species-specific fluorescent dyes. Sillo, Giordano and Gonthier (2018) focused on *H. irregulare* and developed a LAMP method that targets a specific region (cytochrome P450 monooxygenase with haem-binding activity) identified through comparative genomics.[1]		
164	Methods based on PCR targeting mitochondrial DNA have been developed to discriminate the species *H. abietinum*, *H. annosum s.s.* and *H. parviporum* (Gonthier *et al*., 2001; Gonthier, Garbelotto and Nicolotti, 2003). A duplex PCR-based method targeting nuclear and mitochondrial DNA has also been developed to distinguish between *H. annosum s.s.* and *H. irregulare* (Gonthier *et al*., 2007) and to detect hybrids between these two species. In addition, real-time PCR methods have been developed. The internal transcribed spacer (ITS) region was selected by Lamarche *et al*. (2017) to design a set of real-time PCR methods using hydrolysis probes with different levels of specificity to detect the species of *H. annosum s.l.* occurring in North America, *H. irregulare* and *H. occidentale*, and *H. annosum s.s.* Ioos *et al*. (2019) developed multiplex real-time PCR methods for the detection and identification of *Heterobasidion* species infecting conifers in Europe (*H. abietinum*, *H. annosum s.s.*, *H. irregulare* and *H. parviporum*), which can be used simultaneously or individually thanks to probes labelled with species-specific fluorescent dyes. Sillo, Giordano and Gonthier (2018) focused on *H. irregulare* and developed a LAMP method that targets a specific region (cytochrome P450 monooxygenase with haem-binding activity) identified through comparative genomics.[1]	C	*Category : TECHNICAL* **(28) United States of America (5 Apr 2024 4:26 PM)** This is not a simple way to separate these species. However, the process appears sound. The diagnostic process also gets more straightforward if a location is only likely to have one or two possible species in any given area.
165	The above methods can be used for typing isolates and fruiting bodies, as well as environmental (wood) samples. The molecular detection directly from environmental materials represents a powerful tool for a rapid and reliable monitoring of these pathogenic species.	C	*Category : TECHNICAL* **(123) EPPO (29 Apr 2024 5:57 PM)** Are all above methods, including conventional PCR, sensitive to detect the pathogen in the environmental samples?

166	As previously indicated, this diagnostic protocol does not provide a comprehensive review of all molecular methods available for the diagnosis of the species of *H. annosum s.l.* Other molecular tests are available in the literature and other equipment, kits or reagents may be used provided that a verification is carried out.	P	*Category : EDITORIAL* **(124) EPPO (29 Apr 2024 5:57 PM)**
167	**Table 3.** Molecular methods for detection of pathogenic species or groups of species in *Heterobasidion annosum sensu lato*	C	*Category : EDITORIAL* **(126) EPPO (29 Apr 2024 5:57 PM)** Add a column with a reference (author et al. YEAR). Maybe delete the column Section.
167	**Table 3.** Molecular methods for detection of pathogenic species or groups of species in *Heterobasidion annosum sensu lato*	C	*Category : TECHNICAL* **(125) EPPO (29 Apr 2024 5:57 PM)** The assays in Oliva et al 2017 provides another protocol for detecting H. parviporum (and H.annosum) https://www.sciencedirect.com/science/article/pii/S0378112717309398#s0010 Please consider adding this protocol.
167	**Table 3.** Molecular methods for detection of pathogenic species or groups of species in *Heterobasidion annosum sensu lato*	C	*Category : TECHNICAL* **(29) United States of America (5 Apr 2024 4:27 PM)** We can't assess how good these assays are from just the data presented in this table. However, the right combination of these assays does appear to be able to separate these species. Previously in this document it is mentioned repeatedly that these assays were chosen in large part because they are backed by validation data.
168	**Section**	C	*Category : EDITORIAL* **(127) EPPO (29 Apr 2024 5:57 PM)** For the easiness of reading, maybe it would be better to list the reference of the protocol in short (e.g. Bahnweg et al. 2002), in addition to the section, and instead the (source and footnote) indicated in the second column. In this way, the full references at the bottom of the table could be removed as they would appear in the reference list at the end of the protocol anyway.
189	+	C	*Category : TECHNICAL* **(128) EPPO (29 Apr 2024 5:57 PM)** A footnote would be nice here, to describe which taxa are also detected.
244	**3.4.1 Preparation of material**	C	*Category : EDITORIAL*

			(46) Canada (19 Apr 2024 3:32 PM) What material? Should add more info on this subtitle. Tissues?
244	**3.4.1 Preparation of material**	C	*Category : TECHNICAL* **(31) United States of America (5 Apr 2024 4:28 PM)** It appears that these methods should be acceptable for separating out the species of concern.
244	**3.4.1 Preparation of material**	C	*Category : TECHNICAL* **(21) CA (20 Mar 2024 2:37 PM)** As an alternative option, could previously dried mycelium be homogenized after immersion in liquid nitrogen? Please, clarify the question and If it is possible, include the option.
244	**3.4.1 Preparation of material**	C	*Category : TECHNICAL* **(6) Colombia (20 Feb 2024 11:04 PM)** As an alternative option, could previously dried mycelium be homogenized after immersion in liquid nitrogen? Please, clarify the question and If it is possible, include the option.
246	About 100 mg of wood tissue (including sawdust) or fruiting bodies may be disrupted by a tissue pulverizer; various grinding methods can be used, providing they produce a homogenously ground sample (e.g. FastPrep FP120 Cell Disrupter (Qbiogene), TissueLyser (QIAGEN)).[2] Freeze-drying overnight (lyophilization), or pre-freezing in liquid nitrogen or at −80 °C (overnight), can be beneficial for disruption.	C	*Category : TECHNICAL* **(129) EPPO (29 Apr 2024 5:57 PM)** Use of Lysing matrix tubes, or addition of metal beads should be recommended as well, when using tissue pulverizer.
248	Extraction of DNA from fungal cultures (obtained by isolation from wood samples, fruiting bodies or passive spore traps: see section 3.3), can be optimized by culturing isolates in flasks containing a liquid medium (e.g. 2% malt extract (w/v)) at room temperature (18–24 °C) for at least one week. The fungal mycelium is separated from the culture medium (supernatant) by vacuum filtration to dryness on a filter paper, freeze-dried overnight, and subsequently ground in a FastPrep FP120 Cell Disrupter (Qbiogene) or TissueLyser (QIAGEN).[2] DNA can also be extracted from fungal cultures grown on agar Petri dishes. The agar plates can be supplemented with cellophane membranes to facilitate the collection of mycelia.	C	*Category : TECHNICAL* **(132) EPPO (29 Apr 2024 5:57 PM)** move these sentences above extraction from liquid media. I think that for a general diagnostic purposes, researchers will mainly extract DNA from agar plates as liquid cultures provides an additional step. I would add liquid cultures later on as an option, perhaps if someone needs very pure DNA for sequencing etc.
248	Extraction of DNA from fungal cultures (obtained by isolation from wood samples, fruiting bodies or passive spore traps: see section 3.3), can be optimized by	C	*Category : TECHNICAL* **(131) EPPO (29 Apr 2024 5:57 PM)** This can be achieved by easier methods

	culturing isolates in flasks containing a liquid medium (e.g. 2% malt extract (w/v)) at room temperature (18–24 °C) for at least one week. The fungal mycelium is separated from the culture medium (supernatant) by vacuum filtration to dryness on a filter paper, freeze-dried overnight, and subsequently ground in a FastPrep FP120 Cell Disrupter (Qbiogene) or TissueLyser (QIAGEN).[2] DNA can also be extracted from fungal cultures grown on agar Petri dishes. The agar plates can be supplemented with cellophane membranes to facilitate the collection of mycelia.		
248	Extraction of DNA from fungal cultures (obtained by isolation from wood samples, fruiting bodies or passive spore traps: see section 3.3), can be optimized by culturing isolates in flasks containing a liquid medium (e.g. 2% malt extract (w/v)) at room temperature (18–24 °C) for at least one week. The fungal mycelium is separated from the culture medium (supernatant) by vacuum filtration to dryness on a filter paper, freeze-dried overnight, and subsequently ground in a FastPrep FP120 Cell Disrupter (Qbiogene) or TissueLyser (QIAGEN).[2] DNA can also be extracted from fungal cultures grown on agar Petri dishes. The agar plates can be supplemented with cellophane membranes to facilitate the collection of mycelia.	C	*Category : TECHNICAL* **(130) EPPO (29 Apr 2024 5:57 PM)** see previous comment above
251	The DNA from fungal cultures grown on agar Petri dishes can be directly extracted by the “hyphal tip isolation” method (Schweigkofler, O’Donnell and Garbelotto, 2004), modified as described here. Briefly, the fungal mycelium is collected with the tip of a micropipette and suspended in 100 µL of sterile distilled water, frozen in liquid nitrogen for three minutes, thawed at 75 °C, vortexed for one minute, and finally centrifuged for five minutes at 19 000 *g*. The freezing and thawing are repeated three times, with the last thaw extended to 15 minutes. Samples are then centrifuged for five minutes at 19 000 *g* and the supernatant is used as template DNA for PCR.	C	*Category : TECHNICAL* **(133) EPPO (29 Apr 2024 5:57 PM)** Is this suitable for both PCR and qPCR reactions?
251	The DNA from fungal cultures grown on agar Petri dishes can be directly extracted by the “hyphal tip isolation” method (Schweigkofler, O’Donnell and Garbelotto, 2004), modified as described here. Briefly, the fungal mycelium is collected with the tip of a micropipette and suspended in 100 µL of sterile distilled water, frozen in liquid nitrogen for three minutes, thawed at 75 °C, vortexed for one minute, and finally ~~centrifuged~~ microcentrifuged for five minutes at 19 000 *g*. The freezing and thawing are repeated three times, with the last thaw extended to 15 minutes. Samples are then centrifuged for five minutes at 19 000 *g* and the supernatant is used as template DNA for PCR.	P	*Category : EDITORIAL* **(48) Canada (19 Apr 2024 3:36 PM)**
251	The DNA from fungal cultures grown on agar Petri dishes can be directly extracted	P	*Category : EDITORIAL* **(47) Canada (19 Apr 2024 3:36 PM)**

	by the "hyphal tip isolation" method (Schweigkofler, O'Donnell and Garbelotto, 2004), modified as described here. Briefly, the fungal mycelium is collected with the tip of a micropipette and suspended in 100 µL of sterile distilled water, frozen in liquid nitrogen for three minutes, thawed at 75 °C, vortexed for one minute, and finally centrifuged for five minutes at 19 000 *g*. The freezing and thawing are repeated three times, with the last thaw extended to 15 minutes. Samples are then ~~centrifuged~~ microcentrifuged for five minutes at 19 000 *g* and the supernatant is used as template DNA for PCR.		microcentrifuged
252	The LAMP method of Sillo, Giordano and Gonthier (2018) targeting *H. irregulare* can be coupled with a rapid DNA extraction method for mycelia collected from woody spore traps, based on the use of alkaline polyethylene glycol (PEG) (Chomczynski and Rymaszewski, 2006).[1] Briefly, the samples (adhesive tape pieces) are homogenized into a crude macerate using a 10-mm stainless-steel bead in 5 mL plastic tubes containing 2 mL alkaline PEG lysis buffer (Table 4). Tubes are shaken by hand for two minutes, and 1 µL of the ten-fold dilution of the crude macerate is used in the LAMP method (Sillo, Giordano and Gonthier, 2018; EPPO, 2020a).	C	*Category : TECHNICAL* **(182) New Zealand (3 May 2024 5:48 AM)** The authors mention "adhesive tape pieces" . There is no mention of tape in the rest of the protocol so it is unclear when, how and why tape would be used for sampling. This should be either removed from here or additional information about the sampling provided elsewhere.
252	The LAMP method of Sillo, Giordano and Gonthier (2018) targeting *H. irregulare* can be coupled with a rapid DNA extraction method for mycelia collected from woody spore traps, based on the use of alkaline polyethylene glycol (PEG) (Chomczynski and Rymaszewski, 2006).[1] Briefly, the samples (adhesive tape pieces) are homogenized into a crude macerate using a 10-mm stainless-steel bead in 5 mL plastic tubes containing 2 mL alkaline PEG lysis buffer (Table 4). Tubes are shaken by hand for two minutes, and 1 µL of the ten-fold dilution of the crude macerate is used in the LAMP method (Sillo, Giordano and Gonthier, 2018; EPPO, 2020a).	C	*Category : TECHNICAL* **(32) United States of America (5 Apr 2024 4:29 PM)** This DNA extraction protocol, when coupled with LAMP assays, is a commonly used crude DNA isolation method and should be acceptable for this purpose.
266	For all extraction methods, DNA should be stored at −20 °C until use; subsequently, any remaining DNA (i.e. DNA not used for PCR) can be stored at −80 °C or −20 °C.	C	*Category : TECHNICAL* **(184) New Zealand (3 May 2024 5:56 AM)** recommend to say "should be stored at -20C or below". Currently it gives two options: -20 or -80 but there are other ultra-low freezers that are at -70 or -65. More generic wording would be better.
269	***3.4.3.1 Multiplex PCR of Bahnweg* et al. *(2002) targeting* Heterobasidion annosum s.l. *and* Armillaria *spp.***	C	*Category : TECHNICAL* **(33) United States of America (5 Apr 2024 4:31 PM)** This protocol is backed by validation data

			and should be acceptable
270	The *Heterobasidion*-specific primers HET-7a and HET-8a were developed by Bahnweg *et al*. (2002) and combined in a multiplex PCR with the *Armillaria*-specific primers ARM-1 and ARM-2 (Schulze *et al*., 1997) for the simultaneous detection of these two economically-important pathogens of conifers. Both ~~HET-7a~~ HET-7a/HET-8a and ~~HET-8a~~ ARM-1/ARM-2 were derived from ITS sequences. The HET-7a/HET-8a primers are considered genus-specific because differences in ITS sequences between species within *H. annosum s.l.* are very small (Kasuga *et al*., 1993). The specificity of the method was cross-checked against a wide panel of pure cultures from target (*Armillaria* spp. and *Heterobasidion* spp.) and non-target species, including other root- and butt-rot fungi (e.g. *Ilyonectria destructans* (Zinssm.) Rossman, L. Lombard & Crous, 2015), wood decomposers (e.g. *Pholiota squarrosa* (Vahl) P. Kumm., 1871, *Stereum sanguinolentum* (Alb. & Schwein.) Fr., 1838, *Trichaptum abietinum* (Pers. ex J.F. Gmel.) Ryvarden, 1972, *Tyromyces* spp.), ectomycorrhizal fungi (e.g. *Hebeloma mesophaeum* (Pers.) Quél., 1872, *Russula ochroleuca* Fr., 1838) and parasitic Peronosporales (e.g. *Phytophthora* spp., *Pythium* spp.) that may occur in the same environment. The PCR method was verified on environmental wood samples. As little as 1 pg of *Armillaria* DNA in a mixture with 100 ng of *Heterobasidion* DNA still yielded a visible amplicon (Bahnweg *et al*., 2002).	P	*Category : TECHNICAL* **(134) EPPO (29 Apr 2024 5:57 PM)**
276	**Table 5.** Master mix composition, cycling parameters and amplicons for multiplex PCR targeting *Heterobasidion annosum s.l.* and *Armillaria* spp.	C	*Category : TECHNICAL* **(135) EPPO (29 Apr 2024 5:57 PM)** The logics in this protocol and subsequent protocols are not understandable unless it is also mentioned for which enzyme the protocol was optimized as the exact requirement of MgCl2, primers and dNTPs will vary with the enzyme used.
297	DNA polymerase	C	*Category : TECHNICAL* **(49) Canada (19 Apr 2024 3:43 PM)** Should add more information on the type of DNA polymerase as this can have huge impact on the result, can have variation otherwise. Same for qPCR mastermix , also in qPCR, LAMP dye and quencher not always have information. Note, this applies to other paragraphs and tables
304	Annealing	C	*Category : TECHNICAL* **(137) EPPO (29 Apr 2024 5:57 PM)** Annealing and elongation steps are repeated

			under the number of cycles- duplication to delete
304	~~Annealing~~	P	*Category : TECHNICAL* **(136) EPPO (29 Apr 2024 5:57 PM)**
305	~~68 °C for 1 min~~	P	*Category : TECHNICAL* **(138) EPPO (29 Apr 2024 5:57 PM)**
306	Elongation	C	*Category : TECHNICAL* **(140) EPPO (29 Apr 2024 5:57 PM)** Annealing and elongation steps are repeated under the number of cycles- duplication to delete
306	~~Elongation~~	P	*Category : TECHNICAL* **(139) EPPO (29 Apr 2024 5:57 PM)**
307	~~72 °C for 2 min~~	P	*Category : TECHNICAL* **(141) EPPO (29 Apr 2024 5:57 PM)** We believe that this is a mistake as if needed for initial denaturation it would be different than in the cycles - also other cycling parameters elsewhere in the document do not have an Annealing and Elongation in the initial denaturation phase.
327	The PCR products can be visualized by standard agarose gel electrophoresis.	C	*Category : TECHNICAL* **(142) EPPO (29 Apr 2024 5:57 PM)** What is the expected fragment length?
328	***3.4.3.2 Conventional PCR of Gonthier* et al. *(2001) and Gonthier, Garbelotto and Nicolotti (2003) targeting* Heterobasidion abietinum, H. annosum s.s. *and* H. parviporum**	C	*Category : TECHNICAL* **(34) United States of America (5 Apr 2024 4:31 PM)** This protocol is backed by validation data and should be acceptable
330	The master mix and cycling parameters for the PCR are described in Table 6 and the primers are as follows:	C	*Category : TECHNICAL* **(143) EPPO (29 Apr 2024 5:57 PM)** Could you add what primer combination is suppose to detect what species?
371	**Expected amplicons**	C	*Category : TECHNICAL* **(173) Brazil (2 May 2024 7:23 PM)** The expected amplicons' banding patterns are very similar, making the distinction between the species Heterobasidion abietinum (195 bp) and H. parviporum (185 bp) during electrophoresis difficult. The closely overlapping bands can result in ambiguous interpretations.

377	185 bp	C	*Category : TECHNICAL* **(35) United States of America (5 Apr 2024 4:32 PM)** One point of potential concern that does exist with the results of these assays. Some of the PCR product sizes are quite similar in length and could be difficult to distinguish from each other. This could make results difficult to interpret unless the proper controls are used and unless the gel running and imaging systems to be used are well defined
384	The PCR products can be visualized by standard agarose gel electrophoresis.	C	*Category : TECHNICAL* **(144) EPPO (29 Apr 2024 5:57 PM)** What fragment sizes should be expected?
384	The PCR products can be visualized by standard agarose gel electrophoresis.	C	*Category : TECHNICAL* **(22) CA (20 Mar 2024 2:38 PM)** To ensure complete clarity regarding the conditions, it would be appropriate to detail standard gel electrophoresis, including aspects such as voltage, time, and agarose concentration used in the gel. do "Standard" refere to 1.2% agarose, applying a voltage of 100 volts for 20 minutes?
384	The PCR products can be visualized by standard agarose gel electrophoresis.	C	*Category : EDITORIAL* **(15) Colombia (28 Feb 2024 8:20 PM)** To ensure complete clarity regarding the conditions, it would be appropriate to detail standard gel electrophoresis, including aspects such as voltage, time, and agarose concentration used in the gel. do "Standard" refere to 1.2% agarose, applying a voltage of 100 volts for 20 minutes?
385	***3.4.3.3 Conventional PCR of Gonthier* et al. *(2007) targeting* Heterobasidion annosum s.l., H. annosum s.s. *and* H. irregulare**	C	*Category : TECHNICAL* **(36) United States of America (5 Apr 2024 4:33 PM)** This protocol is backed by validation data and should be acceptable
386	A taxon-specific conventional PCR was developed by Gonthier *et al*. (2007) targeting *H. annosum s.s*. and *H. irregulare*; this PCR can also be used to detect hybrids between *H. annosum s.s*. and *H. irregulare*. The DNA was characterized by three sets of PCR primers specifically designed to target one nuclear locus (two	C	*Category : TECHNICAL* **(147) EPPO (29 Apr 2024 5:57 PM)** Has this method not been verified on environmental samples? Section 3.4 mentions all the methods are suitable for environmental samples.

	sets) and one mitochondrial locus in *Heterobasidion* (Gonthier *et al*., 2001). The primers EFaNAPFor and EFaEuPFor were designed on the nuclear elongation factor 1-α (EFA) to exclusively amplify either *H. irregulare* or *H. annosum s.s.*, respectively, when each of them is used in combination with the two *H. annosum* universal primers EFaHaFor and EFaHaRev. The use of both EFa primer sets allows the results obtained by each primer set to be confirmed. The primers Mito 5, Mito 7 and Mito 8 amplify a 121 bp amplicon in the mitochondrial ribosomal operon for *H. irregulare* and one of 158 bp for *H. annosum s.s.* The specificity of the two resulting PCR methods (one targeting the nuclear locus and the other targeting the mitochondrial locus) was verified on 490 isolates of *H. irregulare*, 86 isolates of *H. annosum s.s.* and 6 hybrid isolates between the two species. Hybrid isolates are typed as EU (*H. annosum s.s.*) at the mitochondrial locus and as NA (*H. irregulare*) at the nuclear locus, or vice versa as NA at the mitochondrial locus and as EU at the nuclear locus (see section 1 for hybridization between *H. annosum s.s.* and *H. irregulare*). The methods are 100% specific to the target species; no cross-reaction between species was noted (Gonthier *et al*., 2007).		
386	A taxon-specific conventional PCR was developed by Gonthier *et al*. (2007) targeting *H. annosum s.s.* and *H. irregulare*; this PCR can also be used to detect hybrids between *H. annosum s.s.* and *H. irregulare*. The DNA was characterized by three sets of PCR primers specifically designed to target one nuclear locus (two sets) and one mitochondrial locus in *Heterobasidion* (Gonthier *et al*., 2001). The primers EFaNAPFor and EFaEuPFor were designed on the nuclear elongation factor 1-α (EFA) to exclusively amplify either *H. irregulare* or *H. annosum s.s.*, respectively, when each of them is used in combination with the two *H. annosum* universal primers EFaHaFor and EFaHaRev. The use of both EFa primer sets allows the results obtained by each primer set to be confirmed. The primers Mito 5, Mito 7 and Mito 8 amplify a 121 bp amplicon in the mitochondrial ribosomal operon for *H. irregulare* and one of 158 bp for *H. annosum s.s.* The specificity of the two resulting PCR methods (one targeting the nuclear locus and the other targeting the mitochondrial locus) was verified on 490 isolates of *H. irregulare*, 86 isolates of *H. annosum s.s.* and 6 hybrid isolates between the two species. Hybrid isolates are typed as EU (*H. annosum s.s.*) at the mitochondrial locus and as NA (*H. irregulare*) at the nuclear locus, or vice versa as NA at the mitochondrial locus and as EU at the nuclear locus (see section 1 for hybridization between *H. annosum s.s.* and *H. irregulare*). The methods are 100% specific to the	C	*Category : TECHNICAL* **(146) EPPO (29 Apr 2024 5:57 PM)** I find this section a little confusing. Do PCRs for H.irr and H.ann s.s need to be done separately? Am I right assuming that EFaHaFor and Rev amplify general H.ann s.l., then primer Efa NAPFor with EFaHaRev will amplify H.irr and EFaEUPFor and EFaHaRev H.amm s.s? A table explaining those combinations would be useful

	target species; no cross-reaction between species was noted (Gonthier *et al*., 2007).		
386	A taxon-specific conventional PCR was developed by Gonthier *et al*. (2007) targeting *H. annosum s.s*. and *H. irregulare*; this PCR can also be used to detect hybrids between *H. annosum s.s.* and *H. irregulare*. The DNA was characterized by three sets of PCR primers specifically designed to target one nuclear locus (two sets) and one mitochondrial locus in *Heterobasidion* (Gonthier *et al*., 2001). The primers EFaNAPFor and EFaEuPFor were designed on the nuclear elongation factor 1-α (EFA) to exclusively amplify either *H. irregulare* or *H. annosum s.s.*, respectively, when each of them is used in combination with the two *H. annosum* universal primers EFaHaFor and EFaHaRev. The use of both EFa primer sets allows the results obtained by each primer set to be confirmed. The primers Mito 5, Mito 7 and Mito 8 amplify a 121 bp amplicon in the mitochondrial ribosomal operon for *H. irregulare* and one of 158 bp for *H. annosum s.s*. The specificity of the two resulting PCR methods (one targeting the nuclear locus and the other targeting the mitochondrial locus) was verified on 490 isolates of *H. irregulare*, 86 isolates of *H. annosum s.s*. and 6 hybrid isolates between the two species. Hybrid isolates are typed as EU (*H. annosum s.s.*) at the mitochondrial locus and as NA (*H. irregulare*) at the nuclear locus, or vice versa as NA at the mitochondrial locus and as EU at the nuclear locus (see section 1 for hybridization between *H. annosum s.s.* and *H. irregulare*). The methods are 100% specific to the target species; no cross-reaction between species was noted (Gonthier *et al*., 2007).	C	*Category : TECHNICAL* **(145) EPPO (29 Apr 2024 5:57 PM)** Can information be added on the lenght of the amplicons?
429	**Expected amplicons**	C	*Category : TECHNICAL* **(174) Brazil (2 May 2024 7:26 PM)** The protocol to identify H. annosum s.s. (69 bp) and H. irregulare (71 bp) differs by only three base pairs. More than this minor difference is needed for differentiation in gel electrophoresis, requiring more specific methods (such as sequencing) for a more accurate identification.
435	71 bp	C	*Category : TECHNICAL* **(37) United States of America (5 Apr 2024 4:34 PM)** Because of the small sizes (in base pairs) of two of these products, a diagnostician might have issues using agarose to both visualize these bands, to distinguish between the ones of annosum and irregulare. Similarly, because of the very similar sizes of these

			two PCR products, they could be very difficult to clearly distinguish from each other. This could lead to a misdiagnosis.
438	*Source:* Gonthier, P., Nicolotti, G., Linzer, R., Guglielmo, F. & Garbelotto, M. 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular Ecology*, 16: 1389–1400. https://doi.org/10.1111/j.1365-294X.2007.03250.x	C	*Category : TECHNICAL* **(175) Brazil (2 May 2024 7:28 PM)** We didn't find evidence of the expected band patterns of the amplicons listed in Table 7 in the provided reference by Gonthier et al. (2007) (169 bp for H. annosum s.l., 69 bp for H. annosum s.s., and 71 bp for H. irregulare).
439	**Table 8.** Master mix composition, cycling parameters and amplicons for conventional PCR to target the mitochondrial locus of *Heterobasidion annosum sensu lato*, *H. annosum sensu stricto* and *H. irregulare*	C	*Category : TECHNICAL* **(148) EPPO (29 Apr 2024 5:57 PM)** I thought this PCR was only for H.ann s.s and H.irr, not for H.ann s.l.?
477	121 bp	C	*Category : TECHNICAL* **(38) United States of America (5 Apr 2024 4:34 PM)** Similar to a few of the above comments, because of the sizes of these PCR products, they could be difficult to distinguish from each other unless gel running conditions are well defined, and unless the correct set of positive controls are available for the diagnostician to use.
480	~~*Source*~~*Sources*: Garbelotto, M., Otrosina, W.J., Cobb, F.W. & Bruns, T.D. 1998. The European S and F intersterility groups of Heterobasidion annosum may represent sympatric protospecies. Canadian Journal of Botany, 76: 397–409. https://doi.org/10.1139/b97-185 Gonthier, P., Garbelotto, M., Varese, G.C. & Nicolotti, G. 2001. Relative abundance and potential dispersal range of intersterility groups of *Heterobasidion annosum* in pure and mixed forests. *Canadian Journal of Botany*, 79: 1057–1065. https://doi.org/10.1139/b01-090	P	*Category : EDITORIAL* **(176) Brazil (2 May 2024 7:34 PM)** The reference for the primer Mito 5 (Garbelotto et al. 1998) needs to be included.
481	The PCR products can be visualized by standard agarose gel electrophoresis.	C	*Category : TECHNICAL* **(177) Brazil (2 May 2024 7:37 PM)** The proximity in band sizes can hinder clear distinction between species, suggesting additional methods are needed.
484	***3.4.4.1 Real-time PCR of Lamarche* et al. *(2017) targeting* Heterobasidion annosum s.l., H. annosum s.s., H. irregulare *and* H. occidentale**	C	*Category : TECHNICAL* **(39) United States of America (5 Apr 2024 4:35 PM)** This protocol is backed by validation data and should be acceptable
532	***3.4.4.2 Multiplex real-time PCR of Ioos* et al. *(2019) targeting* Heterobasidion abietinum, H. annosum s.s., H. irregulare *and* H. par viporum**	C	*Category : TECHNICAL* **(40) United States of America (5 Apr 2024 4:35 PM)** This protocol is backed by validation data and should be acceptable

533	Ioos *et al*. (2019) developed real-time PCR detection methods for *H. abietinum*, *H. annosum s.s.*, *H. irregulare* and *H. parviporum*, which can be used individually or simultaneously by using probes labelled with species-specific fluorescent dyes. The primers and probes target a specific region of the DNA replication licensing factor (*Mcm7*) for *H. abietinum*, *H. annosum s.s.* and *H. irregulare*, and the RNA polymerase II large subunit (*RPB1*) for *H. parviporum*. Preliminary attempts to use all four primer–probe sets in a single PCR tube (quadruplex PCR) showed an unacceptable loss of sensitivity. However, it was found that a triplex real-time PCR, using primers and probes for *H. irregulare* and *H. parviporum* plus the FungiQuant primer–probe set of Liu *et al*. (2012) targeting the 18S rDNA of a broad range of fungal species, together with a duplex real-time PCR for *H. abietinum* and *H. annosum s.s.*, could be successfully achieved without compromising the sensitivity of each method.	C	*Category : TECHNICAL* **(149) EPPO (29 Apr 2024 5:57 PM)** Would be good to add in the brackets the purpose of those primers (internal positive control)
592	***3.4.4.3 LAMP*[‡] *of Sillo, Giordano and Gonthier (2018) targeting* Heterobasidion irregulare**	C	*Category : TECHNICAL* **(41) United States of America (5 Apr 2024 4:36 PM)** This protocol is backed by validation data and should be acceptable
594	Sillo, Giordano and Gonthier (2018) assessed the performance of the method, reporting the limit of detection to be about 20 pg of target DNA per reaction, and the time taken to achieve a detection result to be less than 40 minutes. No cross-reactivity was observed with 12 isolates of non-target phylogenetically closely related species, including *H. abietinum*, *H. annosum s.s.* and *H. parviporum*, or with 14 isolates of seven other wood-decay hymenomycetes found in pine trees (*Echinodontium tinctorium*, *Fomitopsis pinicola*, *Fuscoporia torulosa*, *Onnia* spp., *Phaeolus schweinitzii*, *Porodaedalea pini* and *Stereum* spp.). Nine isolates of *H. irregulare* of different origins were also used.	C	*Category : TECHNICAL* **(42) United States of America (5 Apr 2024 4:37 PM)** Is this detection limit number correct? If so, then the limit of detection for this assay might be inadequate to detect the target organism, especially from environmental or metagenomic samples. Many of the DNA based diagnostic methods of which we are used to have detection limits that are capable of detecting picogram or femtogram quantities of target DNA. This may not be a best choice of an assay to deploy
636	[‡] See page footnote 2.	C	*Category : EDITORIAL* **(150) EPPO (29 Apr 2024 5:57 PM)** Add footnotes
640	**3.4.5 Controls for molecular tests**	C	*Category : TECHNICAL* **(43) United States of America (5 Apr 2024 4:37 PM)** This section is well defined and useful
640	**3.4.5 Controls for molecular tests**	C	*Category : TECHNICAL* **(23) CA (20 Mar 2024 2:40 PM)**

			Is It possible to use a positive synthetic control? If so, what would be the expected band size? And If it is possible, include the option.
640	**3.4.5 Controls for molecular tests**	C	*Category : TECHNICAL* **(9) Colombia (20 Feb 2024 11:23 PM)** Is It possible to use a positive synthetic control? If so, what would be the expected band size? And If it is possible, include the option.
655	In Lamarche *et al.* (2017) and Gonthier *et al*. (2007), internal positive controls can include specific amplicons or co-amplification of endogenous nucleic acid, using conserved primers that amplify conserved non-organism target nucleic acid that is also present in the sample (e.g. plant *COX* gene or eukaryotic 18S rDNA).	C	*Category : TECHNICAL* **(151) EPPO (29 Apr 2024 5:57 PM)** Please also consider that the assay of Ioos et al. (2019) includes the Fungiquant assay of Liu et al 2012, targeting the 18S rDNA, and also to be used as an internal positive control, checking the quality of the DNA extract.
656	**3.4.6 Interpretation of results**	C	*Category : TECHNICAL* **(24) CA (20 Mar 2024 2:41 PM)** The suggestion is to consider the possibility of including in Interpretation of results the annexes images of the agarose gel electrophoresis for conventional PCR, LAMP and the qPCR and LAMP curves, along with the corresponding controls. These images would facilitate a more accurate understanding of the expected results for positive or negative samples. It is important to note that this recommendation is subject to the established publication guidelines. It is important to note that this recommendation is subject to the established publication guidelines and the author's discretion.
656	**3.4.6 Interpretation of results**	C	*Category : TECHNICAL* **(10) Colombia (20 Feb 2024 11:25 PM)** The suggestion is to consider the possibility of including in Interpretation of results the annexes images of the agarose gel electrophoresis for conventional PCR, LAMP and the qPCR and LAMP curves, along with the corresponding controls. These images would facilitate a more accurate understanding of the expected results for

			positive or negative samples. It is important to note that this recommendation is subject to the established publication guidelines. It is important to note that this recommendation is subject to the established publication guidelines and the author's discretion.
657	***3.4.6.1 Interpretation of conventional PCR results***	C	*Category : SUBSTANTIVE* **(178) Brazil (2 May 2024 7:55 PM)** It would be worth communicating a potential limitation related to the proximity of band sizes of expected amplicons between different species in PCR methods using specific primers. The techniques described may make distinguishing amplicons of similar sizes challenging, suggesting additional methods such as sequencing are needed.
681	*Heterobasidion annosum s.l.* can be identified by the macro- and micromorphological characteristics of its fruiting bodies, or by its growth characteristics and morphology in pure culture, but experience with the identification of *Heterobasidion* species is required. Even with experience, however, the slight differences and the partial overlapping of morphological traits between *H. annosum s.l.* species often lead to erroneous identifications. Molecular methods therefore provide the most reliable way to accurately identify the pathogen at species level. In some cases, the traditional method using mating experiments with confirmed homokaryotic strains for each species (Korhonen, 1978; Stenlid, 1985; Mitchelson and Korhonen, 1998) can be used as an alternative to molecular methods, but it requires expertise in the interpretation of the results and more time. The conventional PCR and real-time PCR methods described in sections 3.4.3 and 3.4.4 are species specific and are used for detection of the pathogen in infected material, passive spore traps or in pure fungal cultures. DNA sequencing (see section 4.2) can also be performed to confirm the identity of pure fungal cultures of the pathogen.	C	*Category : TECHNICAL* **(152) EPPO (29 Apr 2024 5:57 PM)** see previous comment
683	The fruiting bodies of *H. annosum s.l.* are 1–40 cm across, perennial, very irregular in shape, pileate, resupinate or effused-reflexed, and rubbery in texture (Figure 5, Figure 6 and Figure 8). The top surface (when present) is reddish or dark brown in colour and becomes darker with age. The margin is distinct, thin and white. The context is 0.2−1 cm thick, whitish, and corky to woody. The hyphal system is	C	*Category : TECHNICAL* **(153) EPPO (29 Apr 2024 5:57 PM)** I'm not sure I understand that, does it mean that there are 1 to 3 pores per mm (2?) of surface? Or that the pores are 1 to 3 mm long/deep?

	dimitic and non-agglutinated, with generative hyphae and skeletal hyphae. The lower surface (hymenophore) is labyrinthine or daedaleoid, white or cream coloured, with numerous small irregular pores; the pores are (1–)2–3 per mm and 150–500(–1000) µm in diameter (Figure 8). Tubes are unevenly or distinctly stratified and are 2–10 mm long in each layer. Basidia are 9−13 × 5−7 µm, clavate, four-spored, and without a basal clamp. Cystidia are absent. Basiodiospores are 3.5−5.5 × 3−4 µm, ovoid to broadly ellipsoid, hyaline, thin-walled and slightly asperulate, with a few guttules (CABI, n.d.).		
697	Root collar and primary roots of infected or dead trees, on stem pieces left in the forest and in decay pockets in stumps	P	*Category : TECHNICAL* **(154) EPPO (29 Apr 2024 5:57 PM)** Created by merging other changes together
703	In central Italy, sporocarps can be 30–40 cm, and larger than those of *H. annosum*	C	*Category : EDITORIAL* **(155) EPPO (29 Apr 2024 5:57 PM)** This is the only mention in the protocol of sporocarp, perhaps it should be replaced with fruiting body for consistency. Alternatively sporocarp should be added in bracket earlier on when mentioning fruiting bodies so the reader knows they are used interchangeably.
710	Root collar, along roots and decay pockets within the butt. Also on decayed stem pieces left in the forest.	P	*Category : TECHNICAL* **(156) EPPO (29 Apr 2024 5:57 PM)**
718	In culture, *H. annosum s.l.* can be easily distinguished from other fungi based on its club-like conidiophores with conidiogeneous vesicles 7.5–18(–22) µm diameter (Figure 9; Stalpers, 1978). Asexual spores (conidia), 4–8(–10) × 2.5–5(–6) µm in size, are subglobose to ovoid or lacrymoid, smooth, hyaline, non-septate, thick-walled and without vacuoles (Stalpers, 1978). Colonies grow rapidly on generic agar-based media, reaching 6–8 cm in 7 days; they are white or cream to light buff, ivory yellow or honey yellow, and are sometimes pulverulent because of conidial production (Figure 10). Marginal and aerial hyphae are 1.5–5.5(–8) µm, with clamp connections typically present but rare (Figure 10; Stalpers, 1978). Mycelia originating from germination of single basidiospores are haploid and lack clamp connections.	C	*Category : TECHNICAL* **(158) EPPO (29 Apr 2024 5:57 PM)** I found this confusing, are clamp connections typically present or are they rare?
718	In culture, *H. annosum s.l.* can be easily distinguished from other fungi based on its club-like conidiophores with conidiogeneous vesicles 7.5–18(–22) µm diameter (Figure 9; Stalpers, 1978). Asexual spores (conidia), 4–8(–10) × 2.5–5(–6) µm in size, are subglobose to ovoid or lacrymoid, smooth, hyaline, non-septate, thick-	C	*Category : TECHNICAL* **(157) EPPO (29 Apr 2024 5:57 PM)** Is that diameter?

	walled and without vacuoles (Stalpers, 1978). Colonies grow rapidly on generic agar-based media, reaching 6–8 cm in 7 days; they are white or cream to light buff, ivory yellow or honey yellow, and are sometimes pulverulent because of conidial production (Figure 10). Marginal and aerial hyphae are 1.5–5.5(–8) µm, with clamp connections typically present but rare (Figure 10; Stalpers, 1978). Mycelia originating from germination of single basidiospores are haploid and lack clamp connections.		
720	Cultures of *H. annosum s.l.* can be stored on malt extract agar or potato dextrose agar slopes at 5 °C. DNA can be stored at −80 °C or −20 °C.	C	*Category : TECHNICAL* **(185) New Zealand (3 May 2024 5:59 AM)** recommend to say "should be store at -20C or below". Currently it gives two options: -20 or -80 but there are other ultra-low freezers that are at -70 or -65. More generic wording would be better.
723	The conventional PCR and real-time PCR methods described in sections 3.4.3 and 3.4.4 are species specific and can be used for identification of the pathogen in infected material, passive spore traps or in pure fungal cultures. For *Heterobasidion* isolated in pure culture, sequence analysis of the ITS and EFA regions (see section 4.2.1) is an alternative that can be used for sequence-based, species-level identification.	C	*Category : TECHNICAL* **(159) EPPO (29 Apr 2024 5:57 PM)** See previous comment
724	**4.2.1 ITS and EFA sequencing for species-level identification**	C	*Category : SUBSTANTIVE* **(52) Canada (19 Apr 2024 4:20 PM)** This method alone is not a proper identification protocol, and need further work on phylogenetic analysis after Sanger sequencing. Multi-locus sequencing typing might be a better name for the protocol in application for species-level identification.
725	*Heterobasidion* spp. isolated in pure culture can be identified by the amplification and sequencing of the ITS DNA region (including ITS1, 5.8S and ITS2) with two different primer pairs: ITS1-F and ITS4 or ITS1-F and ITS4-B (White *et al*., 1990; Gardes and Bruns, 1993). The first primer pair, which includes the fungal-selective primer ITS1-F, efficiently amplifies the DNA of ascomycetes and basidiomycetes; the second one, which also includes the phylum-selective (basidiomycetes) primer ITS4-B, efficiently amplifies the DNA of basidiomycetes but results in either no product or an extremely faint product for the DNA of ascomycetes (Gardes and Bruns, 1993). The EFA region (Johannesson and Stenlid, 2003) can be used to further confirm the identification of close species, such as when distinguishing	C	*Category : TECHNICAL* **(50) Canada (19 Apr 2024 3:55 PM)** Should define fully ITS as Internal transcribed Spacer and EFA Elongation Factor Alpha

	between *H. abietinum* and *H. parviporum*. These primer pairs can be used to generate amplification products for sequencing from all species of *Heterobasidion*. The expected amplicon produced using these primers can be generated only from DNA extracted from a pure fungal culture (for the preparation of material and DNA extraction, see sections 3.4.1 and 3.4.2).		
732	**Table 13.** Master mix composition, cycling parameters and amplicons for ITS and EFA sequencing	C	*Category : TECHNICAL* **(160) EPPO (29 Apr 2024 5:57 PM)** Same comment regarding enzyme as previously: The logics in this protocol is not understandable unless it is also mentioned for which enzyme the protocol was optimized as the exact requirement of MgCl2, primers and dNTPs will vary with the enzyme used.
785	**4.2.3 Interpretation of results**	C	*Category : TECHNICAL* **(25) CA (20 Mar 2024 2:42 PM)** The suggestion is to consider the possibility of including in Interpretation of results the annexes images. These images would facilitate a more accurate understanding of the expected results for positive or negative samples. It is important to note that this recommendation is subject to the established publication guidelines. It is important to note that this recommendation is subject to the established publication guidelines and the author's discretion.
785	**4.2.3 Interpretation of results**	C	*Category : TECHNICAL* **(13) Colombia (21 Feb 2024 2:22 PM)** The suggestion is to consider the possibility of including in Interpretation of results the annexes images. These images would facilitate a more accurate understanding of the expected results for positive or negative samples. It is important to note that this recommendation is subject to the established publication guidelines. It is important to note that this recommendation is subject to the established publication guidelines and the author's discretion.
788	There are three additional, useful databases for sequence comparison:	C	*Category : TECHNICAL*

			(161) EPPO (29 Apr 2024 5:57 PM) Should Q-bank be added as well, which is an EPPO curated database? I wonder if a note saying that NCBI is not a curated database could be useful? There is a higher chance of mistakes in those databases
792	For species identification, the sequence should be at least a 99% match to published reference sequences (preferably from type specimens, if available). ~~Dalman~~ Dalman, Olson and Stenlid~~*et al.*~~ (2010) provide GenBank accession numbers for reliable specimens of all *Heterobasidion* species: GenBank accession numbers from FJ627520 to FJ627596 for ITS and FJ627434 to FJ627364 for EFA. Examples are: *H. abietinum* isolate Faf4-2, GenBank accession number FJ627561.1 for ITS and FJ627400.1 for EFA; *H. annosum s.s.* isolate W15, GenBank accession number FJ627596.1 for ITS and FJ627434.1 for EFA; *H. irregulare* isolate MON 111, GenBank accession number FJ627580.1 for ITS and FJ627418.1 for EFA; *H. occidentale* isolate MON 108, GenBank accession number FJ627578.1 for ITS and FJ627416.1 for EFA; and *H. parviporum* isolate Fas1, GenBank accession number FJ627567.1 for ITS and FJ627406.1 for EFA.	P	*Category : EDITORIAL* **(162) EPPO (29 Apr 2024 5:57 PM)** For uniformity, choose either to write the names of three authors that contributed to a single publication in full, or as "et al.". Please check throughout document
804	**8. References**	C	*Category : EDITORIAL* **(179) Brazil (2 May 2024 8:02 PM)** Of the 66 bibliographic references listed, only 12 were published in the last ten years. They led us to question whether they had more recent information and more modern techniques for identifying species
871	**9. Figures**	C	*Category : EDITORIAL* **(180) Brazil (2 May 2024 8:05 PM)** We suggest to add letters on the figures to make a clearer relationship between the photo and what the author wants to demonstrate, both in the body of the text and the figure caption. (e.g.: Figure 1A, Figure 1B)
871	**9. Figures**	C	*Category : TECHNICAL* **(163) EPPO (29 Apr 2024 5:57 PM)** suggestion to add a picture on a wood disc showing the interaction zone between healthy tissue and Heterobasidion affected tissue. SLU Department of Forest Mycology and Pathology has such a picture and I can

			send it though e-mail to EPPO (I was unable to upload it through this IPP)
884	**Figure 3.** Paper-thin mycelium of *Heterobasidion* sp. and cambium necrosis beneath the bark at the tree collar of *Pinus sylvestris* (left). Thicker white mycelium of *Armillaria* sp. beneath the bark at the tree collar of *Pinus sylvestris* (right).	C	*Category : TECHNICAL* **(54) Japan (23 Apr 2024 8:00 AM)** Although Figure 3. compares signs of Heterobasidion sp. and Armillaria sp., it is difficult to recognize the signs of Heterobasidion sp. in the photo. Japan proposes adding enlarged photos of the paper-thin mycelium of Heterobasidion annosum. For example, the image in Figure 8.11 (d) of the following page could be used: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/heterobasidion-annosum
905	**Figure 9.** Conidiophores and conidia of the asexual stage of *Heterobasidion annosum sensu lato* (40× magnification).	C	*Category : EDITORIAL* **(164) EPPO (29 Apr 2024 5:57 PM)** Suggestion to add a scale bar to the microscopic photos in Fig 9 and 10
924	**Figure 10.** White-cream colony of *Heterobasidion annosum sensu lato* (left) and clamp connection (right) (40× magnification).	C	*Category : TECHNICAL* **(166) EPPO (29 Apr 2024 5:57 PM)** Age of the culture observed, and type of culture medium, should be mentioned.
924	**Figure 10.** White-cream colony of *Heterobasidion annosum sensu lato* ~~(left)~~ (right) and clamp connection ~~(right)~~ (left) (40× magnification).	P	*Category : EDITORIAL* **(165) EPPO (29 Apr 2024 5:57 PM)** Alternatively consider changing the position of both photos