

DNA Barcode Curation for Proactive Commercial Plantation Forest-Pest Surveillance

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Executive summary

In June 2024, over 430,000 passengers arrived by air at the New Zealand borders, each representing a small degree of risk to accidental introduction or dispersal of various pest organisms that could have large impacts on our forestry sector. With the current uptake in eDNA as a surveillance method, it is fitting to proactively address the issue of pest surveillance for our exotic and native forestry systems. Online repositories have collated numerous DNA barcodes for pest organisms classified as high-risk, but implementation within watchlists depends on robust curation of barcode reference databases, sufficient taxonomic resolution derived from barcodes, and the successful development of primer sets to broad taxonomic groups

By undergoing the procedure to curate a comprehensive list of DNA barcodes of New Zealand high priority pests for the forestry industry, we can both identify gaps in the data as targets for research objectives, as well as compile a dataset for an early alert system for eDNA surveillance programs. This allows us to then apply this watchlist to community eDNA samples through Wilderlab, while allowing us to identify where further resources should be developed and where barcode information should be regularly checked for updates to continue developing and improving surveillance.

Here, we identified 79 invertebrate species for which the current barcode data is suitable for inclusion within an eDNA watchlist and identified 18 that are non-specific to primer sets in assay panels, or data deficient within the repositories. For microbes, barcodes are known to be less specific, often only identifying organisms to genus level. This resulted in a list of 15 species and 7 genera, developed on the understanding that these are likely to be non-specific and, in some cases, low confidence, needing further work post identification. This list should only be applied as an initial genus ID and not as a definitive pest identifier. On providing these lists, species that are suitable for integration into a forestry specific watchlist on approval by FGLT or the Forest Biosecurity Committee would then need to be fed back to Wilderlab to finalise and implement.

Methods

Developing a curated high-risk forestry-pest list

Two pest lists were curated, one for invasive arthropods and another for pathogens. High risk species were compiled as a combination pest list directly from the forestry industry GIA in New Zealand, Forest Owners Association and the New Zealand Biosecurity (Notifiable Organisms) Order 2016. Organisms that weren't listed from these sources but are directly related to the Forestry industry were also collected from European and Mediterranean Plant Protection Organization (EPPO) alert. Closely related species were identified using the New Zealand Pest Register by searching at genus level. Species were collected regardless of their status in New Zealand, as some pest organisms have restricted range or unknown distribution. Pseudonyms were also identified for species where relevant, to increase the accuracy of results.

Barcodes were compiled initially from the Barcode of Life Data (BOLD) global database (Ratnasingham & Hebert, 2013; Ratnasingham et al., 2024) using the barcode index number (BIN) system (Ratnasingham & Hebert, 2013). For those species that weren't present in BOLD, or had low infrequent barcodes (<5), additional searches were conducted in GenBank or in the literature. Species were searched within the respective repositories, and barcode sequence files of the barcodes were then downloaded. Metadata was collected based on the number of sequences present for each. For arthropods, this was specific to COI, whereas for pathogens, various barcodes (COI, ITS, 16S, 18S).

Computational PCR to test barcodes with mismatches

Summary

The primer sequences we had contained degeneracies. This means, that instead of the standard pair matching in DNA code, we can match multiple variations. We often use this when trying to identify regions with known variation that we can capture between species. To test these primers on our sequences of interest, we ran a computational simulation of a PCR (polymerase chain reaction), to identify regions that match these primers in the DNA of target pest species. To make sure we captured the full diversity of the barcodes for every species available, we allowed for up to four mismatches on a loose PCR simulation – i.e. up to four of the DNA codes could not match the sequence of the primer, if they weren't in a critical region of the primer binding. If there were more than 4 or they were in a critical area, they were removed. Another simulation was run with only up to one mismatch per primer, which is a more successful and reliable scenario for high confidence.

Two scenarios were ran using primers for aquatic and terrestrial species as is used in Wilderlab assay panels. After results were collected, duplicates were removed and a list of barcodes for each species was assembled that was tested for how unique it was to its species or genus.

Detailed methods

Primer sequences with degeneracies used for eDNA biodiversity survey panels in New Zealand were acquired from Wilderlab (Table 1). Computation PCRs were set up to run on a custom python script that considers ambiguity codes for nucleotide degeneracies and primer mismatches for the forward and reverse primers. To be less conservative and capture as much diversity in the amplified regions as possible, mismatches were set to a maximum of four per primer if they did not reside on the 3' end, to maximise binding. Outputs were then screened and manually removed if there were more than three mismatches per primer. As COI primers designed for aquatic invertebrates were found to also capture some terrestrial diversity, two computational PCRs were run to use the XIF and XIR, as well as the XIF and XNR combination to identify differences between panels. Outputs files were then sorted by frequency and filtered to remove duplicate sequences to leave a finalised list of sequence amplicons per species.

Table 1 Primers used to test barcodes with computational PCR in this study

Region	Name	Forward primer	Reference
Cytochrome oxidase I <i>(aquatic invert)</i>	XIF		Modified: (Leray et al., 2013)
	XIR		Modified: (Wilkinson et al., 2024)
<i>(terrestrial invert)</i>	XNR	TANACYTCNGGRTGNCCRAARAAYCA	(Geller et al., 2013)
Fungal ITS1	GFF	GGAAGTAAAAGTCGTAACAAGG	(White et al., 1990)
	GFR	CAAGAGATCCGTTGYTGAAAGTK	(Taberlet et al., 2018)
Microbial 16S	UMF	GGATTAGATACCCTGGTA	(Morey et al., 2006)
	UMR	CCGTCAATTCMTTTRAGTTT	(Lane et al., 1985)
Eukaryote/prokaryote 18S	BUF	TTGTACACACCGCCC	(Amaral-Zettler et al., 2009)
	BUR	CCTTCYGCAGGTTACCTAC	(Amaral-Zettler et al., 2009)

Five PCR conditions were explored (2 invertebrates; 3 microbial). Metadata was collated from each output to report the number of amplicons found with the associated number of primer mismatches, mismatch location (if relevant), number of usable barcodes and the number of unique barcodes. Sequence identity was then calculated between all the unique barcodes within a species to determine similarity, before specificity was assessed. Unique barcodes were then checked using *NCBI BLASTn* (Available: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>; Johnson et al. (2008)) for sequence

similarity, as well as within the BOLD systems database search tool. Similarity hits higher than the interspecies similarity determined by *Clustal Omega* (Sievers et al. 2011) – a program use for multi-sequence alignments – were reported. By aligning barcode sequences, we can then compare similarity as a representation of % base pairs shared. Often, species level similarity is defined as >95% similarity, and searches in BOLD database use 94% in their species search tool.

Finally, species were clustered into taxonomic group (sub family i.e. Lymantriinae; genus i.e. *Ips*, *Hylastes*). Percentage identify was calculated between each species to identify whether amplification of the region would result in non-specific amplification from other closely related species group using the species cut off level of >95%.

Results

Invertebrates

Testing PCR panel conditions

Here, two PCR panels were tested as per the Wilderlab testing assays: COI aquatic invertebrate (using recently updated primers) and COI terrestrial invertebrates. These panels share the same forward primer and have an alternate reverse primer. We found that the aquatic invertebrate COI panel more broadly detected amplification of terrestrial invertebrate barcodes in computation PCRs when PCR mismatches were loose (up to 4 per primer, Supplementary File 1, Table S1). In some instances, however, the terrestrial invertebrate primers (n=8) showed an increased number of barcodes matches at fewer mismatches per primer (Supplementary File 1, Table S2, highlighted in green). In the instance of *Tomicus piniperda* – a Biosecurity notifiable organism – two mismatches using the aquatic COI primers were located on the 3' end of the reverse primer. However, when the terrestrial primer was used, 12 sequences have no mismatches, showing that in this instance, the terrestrial invertebrate primer set would be most beneficial for identification. This is a good indicator that use of both primer sets would have better capture results. As most of these species with mismatches using the aquatic primers generally had sufficient capture at one or two mismatches per primer, therefore the benefit in using this panel would be minimal. Therefore, we compiled the following data primarily for the aquatic, updated COI panel as it had the broadest range of selection.

Targeted amplification – loose and strict mismatches

We ran the computation PCR at both loose (allowing 4 mismatches per primer) and strict (allowing up to 1 mismatch per primer). We initially collated results at the loose threshold to determine a broad range of barcodes and test the specificity of these within the dataset that may amplify if the

DNA was abundant, though likely amplification would not occur in a true scenario. We also recorded how many barcodes with 0-4 mismatches per primer there were (Supplementary File 1, Table S1). We then constrained the dataset to those that only had 1 mismatch per primer (a more likely scenario), to identify species where we were confident amplification would occur (Supplementary File 1, Table S3). This would then set the basis for the watchlist. For the strict threshold of 1 mismatch per primer, we identified 67 species that were likely to be amplified. We listed these as high or lower confidence based on the number of available barcodes per species. A further 12 species were amplified with looser thresholds (maximum of 2 mismatches per primer) and therefore were included in the list with lower confidence (Table 3), resulting in a list of 79 species and subspecies suitable for a watchlist.

Gaps in the database

We have identified seventeen species (17.5%) of our species list that do not have the COI barcode coverage or the amplification with current primers (to a loose mismatch level) within the repositories for integration into a watchlist. Of these, one is a Biosecurity notifiable organism under the 2016 order (*Melanophila californica* (pseudonym, *Phaenops californica*), no sequences). Two species on the forestry high risk pest list had no amplification using current primer sets (Table 2), indicating further degeneracies or primer sets should be used. This was found using both COI primer sets for aquatic and terrestrial invertebrates. Further literature searches were undertaken to identify any further COI barcodes for these species. For *Marchalina hellenica*, research using the mitochondrial COI was last published in 2011 (Bouga et al., 2011) and in 2023 (Eleftheriadou et al., 2023), resulting in the three barcodes used here. For the three barcodes, four mismatches were found in the reverse primer and one on the forward primer, suggesting that changes to the reverse primer would need to be made for successful amplification. In this instance, it is suggested that either development of species-specific primers, or more resources for barcodes would be beneficial for filling this gap. In the case of *Phaulacridium vittatum*, the barcodes in the repository are from the 3' end of the COI gene, which is much less represented in databases and less used for metabarcoding. In this case, sequencing of the 5' end of the gene would be required to get sufficient coverage.

Table 2 Species where no usable barcodes were found within the repositories

<i>Species</i>	Status	Source	Reason
<i>Anoplophora nobilis</i>	Not present	MPI - unwanted, closely related	Not in BOLD

<i>Hylastes gracilis</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Hylastes macer</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Ips emarginatus</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Ips lecontei</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Ips paraconfusus</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Ips plastographus</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Lymantria ampla</i>	Not present	MPI - unwanted, closely related	No amplification
<i>Lymantria juglandis</i>	Not present	MPI - unwanted, closely related	Not in BOLD
<i>Lymantria mathura</i> <i>subsp. Aurora</i>	Not present	MPI - unwanted, closely related	Not in BOLD
<i>Marchalina hellenica</i>	Not present	FGR - high risk list, EPPO	No amplification
<i>Melanophila californica</i> (<i>Phaenops californica</i>)	Not present	Biosecurity notifiable organisms order 2016	Not in BOLD
<i>Orgyia basalis</i>	Not present	MPI - unwanted, closely related	Not in BOLD
<i>Orgyia gonostigma</i>	Not present	MPI - unwanted, closely related	Not in BOLD
<i>Orgyia hopkinsi</i>	Not present	MPI - unwanted, closely related	Not in BOLD
<i>Orthotomicus erosus</i>	Not present	Biosecurity notifiable organisms order 2016	No amplification
<i>Phaulacridium vittatum</i>	Not present	FGR - high risk list	No amplification

Barcode specificity

To test the specificity of barcodes to the species level, individual barcodes were queried against the NCBI nucleotide database using *BLASTn* to identify the top hits that were above the cut off for a loose species level (set at 95% identity). The same was done by checking the barcodes again within the BOLD database, to find overlapping hits. Approximately 12 species were identified as having potential issues with taxonomic specificity (Table 3). Those species that had issues with specificity were still included within a recommended watchlist if barcodes existed, but with the caveat that further exploration would be needed to confirm identification. As *Anoplophora malaisica* had one barcode that was the same as *A. chinensis*, we only included one species in the recommended watchlist (included as low confidence), with the caveat that it is non-specific.

Table 3 Species where there were potential overlaps in barcode specificity

Species	Status in NZ	Source	Taxonomic disparity
<i>Anoplophora horsefieldii/ malaisica</i>	Not present	EPPO alert list 2025/ MPI	<i>Anoplophora malaisica</i> has one barcode, the same as <i>A. chinensis</i> . <i>A. horsefieldii</i> had 2 barcodes but weren't species specific
<i>Euzophera semifuneralis</i>	Not present	EPPO alert list 2025	BOLD: 100% <i>Euzosphaera semifuneralis</i> , 100% <i>E. habrella</i> , 95.28% <i>Euzosphaera</i> sp.
<i>Ips cembrae/ typographus</i>	Not present	MPI - unwanted, closely related	The same barcode as <i>Ips typographus</i> COI alt2.
<i>Lymantria beatrix</i>	Not present	MPI - unwanted, closely related	BOLD: <i>Lymantria beatrix</i> 100%, <i>Lymnatria marginata</i> 100%, <i>L. hauensteini</i> 99.21%, <i>L. atemeles</i> 98.43%
<i>Nysius vinitor</i>	Not present	Brendan- high risk pest list	BOLD: <i>Nysius</i> sp 100%, <i>Nysius vinitor</i> 100%, <i>N. caledoniae</i> 100% Genbank: <i>N. plebius</i> 99.21%
<i>Orgyia postica/ australis</i>	Not present	Brendan- high risk pest list	BOLD: <i>Orgyia postica</i> 100%, 99.21% <i>Orgia</i> sp, 99.21% <i>Orgyia australis</i>
<i>Pineus strobi</i>	Not present	Brendan- high risk pest list	BOLD: 98.43-100% <i>Pineus strobi</i> , 99.21 <i>P. orientalis</i> , 99.21% <i>P. pini</i>
<i>Pseudips mexicanus</i>	Not present	EPPO Alert list 2025/ MPI - unwanted, closely related	Some hits in BOLD coming back as 100% <i>Pseudips concinnus</i> .
<i>Trachymela sloanei</i>	Present	EPPO alert list 2025	ID only to genus level in bold

To identify any issues of specificity within species group (i.e., Lymantriinae, *Ips*, *Hylastes* and *Cinara*), percentage identify (%ID) the base pair similarity between two sequences, was used. We calculated the mean base pair similarity within each species to show the variation found within a species, as well as within a broader group such as genus or family. By calculating this, we can see whether there is enough variation between species to correctly identify a species from environmental samples, or whether there is a risk of misidentification if they are too similar. *Clustal OMEGA* was used to determine the inter species %ID by generating a values matrix and recording the range. Once all barcodes were identified, %ID between species was calculated and a heatmap of the mean was produced for each species group (Figure 1A-D). No overlap was found between most species, except for *Lymantria dispar* and its subspecies *asiatica*, *japonica* and *dispar* (Figure 1A), where this was expected and used as a control. From this, we identified potential specificity overlap with *Lymnatria obsfucata*. We also confirmed potential non-specificity for *Ips cembrae/ Ips typographus*, and *Orgyia australis/O. postica* (as well as *O. papuana*). The most problematic group were *Anoplophorus* species,

where more similarity was found between the barcodes. However, this could be an artifact of there being few and less unique/ specific barcodes for some of the species (including species *A. horsefieldii* and *A. malaisica*). This means that when used within a surveillance watchlist, further confirmation would need to be undertaken to determine species such as identifying an organism visually, or targeted species-specific PCR. Overall, most closely related species were different enough in mean barcode %ID that it wouldn't be expected to cause misidentification.

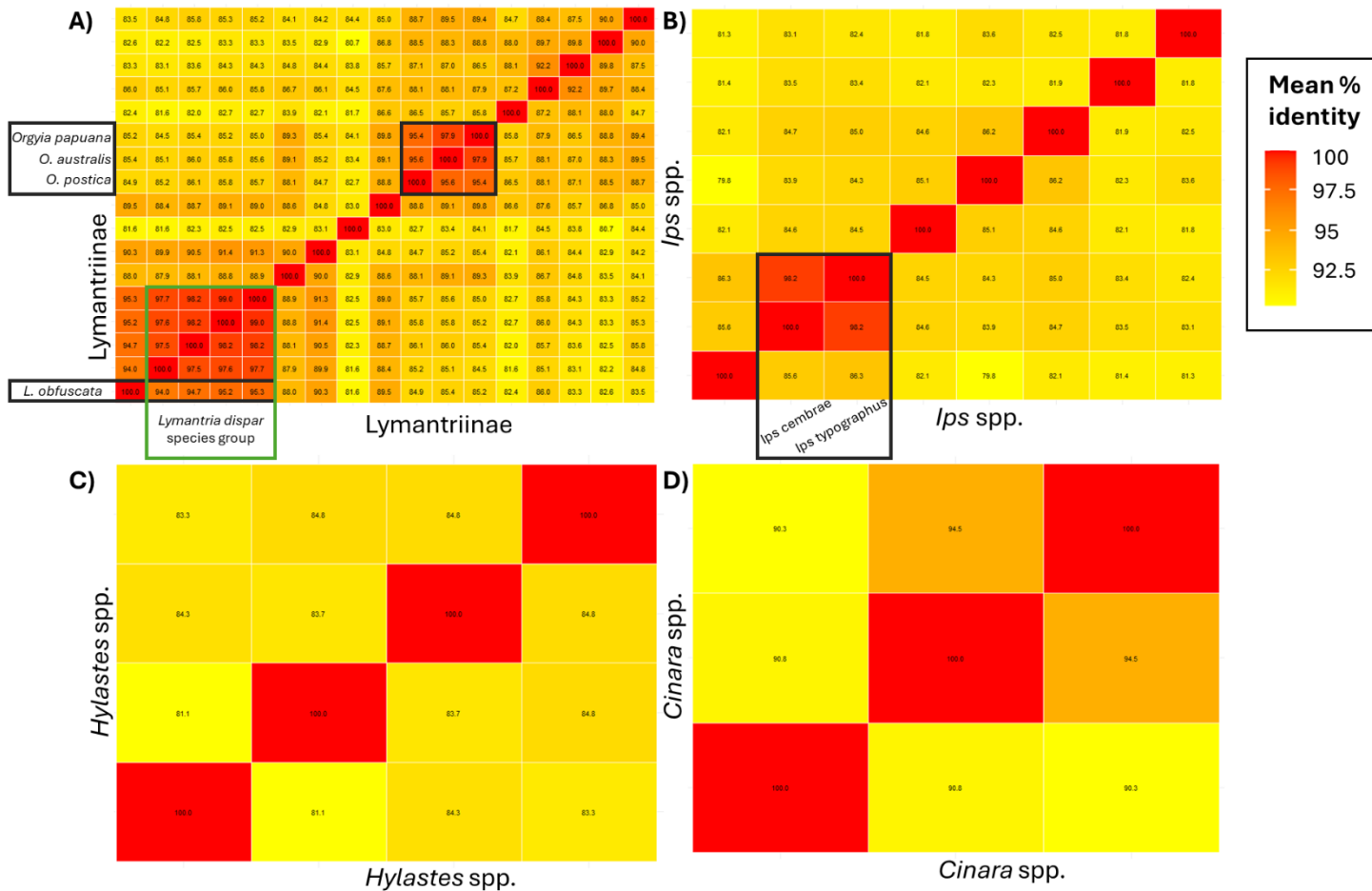


Figure 1 Percentage identity between species groups. Black box denotes where the %ID between two species is higher than the cut off (>95%) used here for species. Green box shows *Lymantria dispar* and its subspecies. A) Lymantriinae – including all *Lymantria* and subspecies, *Orgyia* and *Teia anartoides* (n=17); B) *Ips* species (including *Pseudips*; n=8); C) *Hylastes* species (n=4) and D) *Cinara* species (n=3).

Final invertebrate list

With the above observations considered, the below list is the final list of invertebrates suitable for a Forestry specific surveillance watchlist (Table 3).

Table 3 Final COI invertebrate list suitable for a watchlist in NZ contains 79 species and subspecies. Bold indicates those already present in New Zealand

¹ Forestry high-risk list

² FFNZ unwanted pest list

High confidence			
<i>Agrotis radians</i> ¹	<i>Anoplophora glabripennis</i> ^{2,4}	<i>Anoplophora macularia</i> ³	<i>Arhopalus rusticus</i> ¹
<i>Buprestis aurulenta</i> ⁴	<i>Cinara atlantica</i> ¹	<i>Cinara cupressi</i> ²	<i>Cinara pinivora</i> ¹
<i>Cladius grandis</i> ²	<i>Coptotermes acinaciformis</i> ^{1,4}	<i>Corythucha ciliata</i> ²	<i>Dendroctonus valens</i> ⁴
<i>Ectropis excursaria</i> ¹	<i>Epiphyas caryotis</i> ¹	<i>Euwallacea fornicatus</i> ²	<i>Euzophera semifuneralis</i> ⁵
<i>Halyomorpha halys</i> ²	<i>Hylobius abietis</i> ^{2,4}	<i>Hylurgus ligniperda</i> ¹	<i>Hyphantria cunea</i> ^{2,4}
<i>Ips cembrae</i> ³	<i>Ips pini</i> ³	<i>Ips sexdentatus</i> ³	<i>Ips typographus</i> ³
<i>Leptoglossus occidentalis</i> ¹	<i>Lymantria dispar</i> ^{1,4}	<i>Lymantria subsp. Dispar dispar</i>	<i>Lymantria subsp. dispar japonica</i>
<i>Lymantria subsp. dispar asiatica</i>	<i>Lymantria mathura</i> ³	<i>Lymantria monacha</i> ^{1,4}	<i>Monema flavescens</i> ⁵
<i>Monochamus alternatus</i> ^{1,4}	<i>Nysius vinitor</i> ¹	<i>Orgyia antiqua</i> ¹	<i>Orgyia australis</i> ¹
<i>Orgyia leucostigma</i> ³	<i>Orgyia postica</i> ³	<i>Pineus strobi</i> ¹	<i>Rhagium inquisitor</i> ¹
<i>Rhyacionia buoliana</i> ²	<i>Sirex noctilio</i> ¹	<i>Thaumetopoea pityocampa</i> ^{2,4}	<i>Coptotermes formosanus</i> ⁴
<i>Xanthogaleruca luteola</i> ²	<i>Xylosandrus crassiusculus</i> ²	<i>Monochamus</i> ⁴	<i>Bracharoa</i> (synonym <i>Orgyia</i>) <i>mixta</i> ¹
<i>Tomicus piniperda</i> ⁴	<i>Hylastes ater</i> ¹	<i>Anoplophora horsfieldii</i> ⁵	<i>Hylastes nigrinus</i> ³
<i>Ips grandicollis</i> ¹	<i>Orgyia thyellina</i> ^{2,4}	<i>Pissodes nemorensis</i> ⁴	
Low confidence			
<i>Anoplophora chinensis</i> ³ (same barcode as <i>A. malasiaca</i>)	<i>Cryptotermes brevis</i> ^{2,4}	<i>Urocerus gigas</i> ¹	<i>Orgyia prisca</i> ³
<i>Heterobostrychus aequalis</i> ²	<i>Lymantria beatrix</i> ³	<i>Neodiprion lecontei</i> ⁴	<i>Ips lecontei</i> ³
<i>Metura elongatus</i> ¹	<i>Paropsis charybdis</i> ¹	<i>Hylastes opacus</i> ¹	<i>Eulachnus agilis</i> ¹
<i>Hylastes attenuatus</i> ¹	<i>Hypothenemus eruditus</i> ¹	<i>Platypus semigranosus</i> ¹	<i>Teia anartoides</i> ^{3,4}
<i>Orgyia papuana</i> ³	<i>Ips confusus</i> ³	<i>Pseudips mexicanus</i> ³	<i>Lymantria xylinea</i> ³
<i>Ips duplicatus</i> ³	<i>Lymantria obfuscata</i> ³	<i>Austracris guttulosa</i> ¹	<i>Trachymela sloanei</i> ⁵

³ Closely related species - MPI

⁴ Biosecurity notifiable organisms order 2016

⁵ EPPO alert list 2025

Microbes:

Of the 72 species, final barcodes were identified for 15 (where barcodes were derived from the target species) (Table 4). Seven diseases were caused by genus level pathogens, so genus searches were primarily used here, resulting in usable barcode detection for seven of eight (85%). Twenty-four genera of known pathogens however had no amplification (44%). PCR efficacy for genera was low, with less than 10% barcodes having 'effective' primer hits with few mismatches, meaning a major problem was with the primer sets used. Full database searches and results for species and genera can be found in Supplementary File 1, Tables S4 and S5.

Many of the barcodes had a high degree of similarity with other species within the same genus, which is unproblematic for those that are introduced/ multiple pathogens introduced/ originating from New Zealand. Specificity of 'top hit' results in BLASTn often correlated with increased unique barcodes in the search parameters. Fewer barcodes meant less genus specific hits. Non-specific hits outside of the genus were reported in low levels and were often associated with closely related pathogens (e.g., *Ophiostoma* spp. and *Sporothrix* spp). Problems would arise for genera that have high abundance in the environment, such as *Fusarium* or *Pseudomonas*, where inability to define to species level would likely result in false positives from other non-problematic species to the industry. In this instance, there were 11 species level barcodes for *Fusarium oxysporum* that had 100% hit at species level in GenBank BLASTn .Overall, microbial detection at species level from eDNA is currently difficult, due to the similarity between species in the 16S/ITS/18S regions and overall lack of specificity. A potential watchlist was compiled of species of concern for New Zealand forestry, though problems with none-specificity with genera such as *Fusarium* is expected.

Non-specificity and summary of use of microbial list

For microbes, the use of DNA barcoding for identification from environmental samples often does not provide enough for implication in a surveillance watchlist, which is dependent on sufficient taxonomic resolution from the target gene, and the completion of robust reference databases (Zaiko et al., 2018). This is especially an issue in areas where high levels of diversity are found for a particular species/ genus (relevant here where indicated in Table 4; bolded species show those already in New Zealand) or where the barcodes commonly used do not provide enough resolution and are similar between species or groups, requiring further analysis with multiple target genes not included in assay panels (such as for *Fusarium*, where multi-locus ID of Tef1, RPB2 and BT2 is needed for species level ID; Al-Hatmi et al., 2016). Barcoding with common barcode regions is often only a reliable identification tool for species that are from monotypic genus (those including a single

species), such as *Austropuccinia psidii* (myrtle rust; pseudonym *Puccinia psidii*), where results here show that barcodes are unique and can identify to species level. For example, the 16S gene in its entirety (1500bp) often used to identify bacteria has been shown to identify to genus level in over 90% of instances, whereas species level is significantly lower (Janda & Abbott, 2007).

Therefore, the below species/genus level list is tentatively presented to provide an outline for species where this information exists, whilst not necessarily providing the specificity found in the invertebrate COI list. As a monotypic genus, *Austropuccinia psidii* could be used as a test case to identify applicability of the microbial watchlist to eDNA datasets.

Final species-level microbe list

Table 4 Low confidence microbial list potentially suitable for a watchlist in NZ.
Bold indicates those already present

Species level:			
<i>Austropuccinia psidii</i> ¹	<i>Botryosphaeria dothidea</i> ²	<i>Bursaphelenchus xylophilus</i> ⁴	<i>Collectotrichum gleosporioides</i> ²
<i>Fusarium circumnatum</i> ^{1,4}	<i>Fusarium oxysporum</i> ⁵	<i>Fusarium solani</i> ⁵	<i>Gremmeniella abietina</i> ¹
<i>Heterobasidion annosum</i> ^{1,4}	<i>Lecanosticta acicola</i> ⁴	<i>Phaeoacremonium rubrigenum</i> ²	<i>Phaeolus schweinitzii</i> ²
<i>Teratosphaeria eucalypti</i> ²	<i>Xylella fastidiosa</i> ³ (16S)	<i>Colletotrichum acutatum</i> ⁴	<i>Rhizosphaera kalkhoffii</i> ²
Genus level:			
<i>Leptographium</i> ²	<i>Lophodermium</i> ²	<i>Rhizosphaera</i> ²	<i>Sarcostroma</i> ²
<i>Sporothrix</i> ²	<i>Marssonina</i> ²	<i>Cladosporium</i> ²	

¹ Biosecurity notifiable organisms order 2016

² NZFFA disease list

³ MPI

⁴ Forestry high-risk list

⁵ Closely related species

Outcomes

We have generated a species-specific list of high-risk priority insect pests where barcode data is complete, and suitable enough to be integrated into an eDNA surveillance list. This will be provided to Wilderlab to integrate a forestry specific surveillance watchlist to alert for the presence of high-risk pests in community eDNA samples. We have also generated a list of microbes to genus level where surveillance could be undertaken, on the understanding that this will be less specific and in often cases, low confidence. Implementation of a microbial watchlist comes with the caveat that for a lot of species, species-level ID is not possible, and an abundance of localised species could create false positives.

In addition to developing a list of species where the data is complete, we have identified which high-risk, or priority pest organisms either do not amplify with the current eDNA protocols or have insufficient barcodes within the repository to accurately confirm a positive ID in environmental sampling. This information can then be further used and implemented as a watchlist for high-risk forestry pests when finalised and approved by FGLT and provided to Wilderlab.

Finally, we provided the list of full barcodes that passed loose thresholds to Wilderlab, who have integrated these into their database, resulting in updates to 29 eDNA records across 7 barcode sequences and 6 species, increasing the robustness of results from eDNA panels and identification of pest organisms. This further aids the curation of complete databases with curated barcodes, which improves subsequent identification of pest species should they be detected within samples.

Future research

We have identified gaps in the barcodes from repositories that need to be filled before a complete DNA barcode watchlist can be compiled for all high-risk invertebrate species. In the case of microbes, ideally secondary assays to ID at species level would be required to confirm species identification, many of which exist within the literature. Further work needs to continue within this space to develop the repositories and identify a broad barcode suite that can differentiate to species level. Finally, continuously developing primer sets and assay panels will aim to capture the remaining organisms that are thus far, filtered out without amplification.

References

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	Table S1 Computational PCR results using the aquatic invertebrate COI primers from Wilderlab, showing the number of PCR primer mismatches (per primer).																					
2																						
3	low frequency barcodes		Number of allowed PCR primer mismatches										Within family specificity check					Accuracy		Specificity		
4	#	Species	Status	Source	Sequences*	0	1	2	3	4	no amp found	Mismatch location	Usable barcodes ? [†]	% ident in species	% ident in family	Mega alignment	Seq ID	Consensus barcode?	BOLD search	nBLAST	final number of barcodes for list	notes for specificity of COI/ primer mismatches
5	1	<i>Agrotis radians</i>	Not present	Brendan - high risk list	5	4	4	4	4	4	1	NA	4	98.43-100%		4 seq	98.43-100% (2bp)	N	no hits	100% <i>Agrotis radians</i> ; 99.21% <i>Agrotis munda</i>	2	
6	2	<i>Austracris guttulosa</i>	Not present	Brendan - high risk list	12	0	0	6	10	10	2	2 x 3'; rev	0	99.21-100%	NA	6 seq	100% (1 seq 99.21: 1bp)	N	<i>Austracris guttulosa</i> 99.21-100%; <i>Valanga irregularis</i> 98.43%; <i>Nomadacris apicera</i> 96.06%	2 (maybe 3' mismatch)	3' mismatches at bp 7 and 9	
7	3	<i>Bracharoa mixta</i> (synonym <i>Orygia mixta</i>)	Not present	Brendan - high risk list	1	0	1	1	1	1	0	fwd	1	-		1 seq	100%	Y	100% <i>Bracharoa mixta</i> , 92/97% <i>Elaphria</i>	1	Only 1 available barcode	
8	4	<i>Heterobostrychus aequalis</i>	Not present	FFNZ unwanted pest list	5	0	3	3	3	3	2		3	100%		3 seq	100%	N	100% <i>Heterobostrychus aequalis</i> , 100% <i>heterobostrychus</i>	3	BOLD list has barcode hit to genus with some hit for species	
9	5	<i>Hylastes ater</i>	established - high risk	Brendan - high risk list	5	1	5	5	5	5	0	3'-middle; rev	3	89.76-100%		3 seq	89.76-100% (10bp)	N	98.43-100% <i>Hylastes ater</i> ; 92% <i>Hylastes brunneus</i>	3	3' end mismatch on 2 sequence at bp 9	
10	6	<i>Hylastes attenuatus</i>	Not present	Brendan - high risk list	13	0	0	8	9	10	3	2x3';fow	8	92-100%	81.1-84.8% (including <i>Hylastes opacus</i>)	8 seq	100%	Y	100% <i>Hylastes attenuatus</i> ; 99.21% <i>Hylastes angustatus</i>	1	3' mismatch but not within first 10bp so have included, at basepairs 10 and 13 from 3'	
11	7	<i>Hylastes gracilis</i>	Not present	Closely related high risk pest	2	0	0	0	0	1	1	4x rev	0	-		-	-	-	-	-	0	all 4 mismatches are on the reverse primer
12	8	<i>Hylastes macer</i>	Not present	Closely related high risk pest	1	0	0	0	0	0	1	NA	0	-		-	-	-	-	-	0	no primer matches with mismatch cutoff
13	9	<i>Hylastes nigrinus</i>	Not present	Closely related high risk pest	5	1	4	4	4	4	1	5' rev	4	94-100%		4 seq	94.49-100% (7bp)	N	cluster 1&2: 99.21-100% <i>Hylastes nigrinus</i>	3		
14	10	<i>Hypothenemus eruditus</i>	Not present	Brendan - high risk list	15	1	5	14	14	14	1	5' rev; 5' for	14	76.38-100%	NA	14 seq	76.38-100%	N	100% <i>Hypothenemus eruditus</i> ; 92% <i>Hypothenemus</i> sp	2	This will be incorrect, % identity for most is too low, seems to be two clusters. Have two sequences for these clusters - other four have not included as there is only one of each/ some have come back as other species (<i>Stelidota geminata</i> / no hits in BOLD)	
15	11	<i>Ips confusus</i>	Not present	Closely related high risk pest	3	0	0	1	1	1	2	5' f, mid r	1	100%		1 seq	100%	Y	100% <i>Ips confusus</i>	1		
16	12	<i>Ips duplicatus</i>	Not present	Closely related high risk pest	15	0	0	7	7	7	8	2f 1 rev, 5'	8	99.21-100%		7 seq	99.21-100%	N	100% <i>Ips duplicatus</i> ; 97.64-99.21% <i>Ips midius</i> , 90.55% <i>Ips hauseri</i>	3	2 mismatches on each barcode so may be pushing it	
17	13	<i>Ips emarginatus</i>	Not present	Closely related high risk pest	2	0	0	0	0	0	2	NA	0	-		-	-	-	-	-	0	
18	14	<i>Ips grandicollis</i>	Not present	Brendan - high risk list	11	1	5	5	5	5	6	3' rev	6	95-100%		5 seq	100% (1seq 95%)	N	100% <i>Ips grandicollis</i> 100% (one 95%)	2	mismatch 8bp from 3' end of reverse primer	
19	15	<i>Ips lecontei</i>	Not present	Closely related high risk pest	2	0	0	0	0	0	2	NA	0	-		-	-	-	-	-	0	
20	16	<i>Pseudips mexicanus</i>	Not present	Closely related high risk pest	29	0	5	11	11	11	18	3' rev	11	89.76-100%		11 seq	89.76-100%	N	100% <i>Pseudips mexicanus</i> 95.24-100%; <i>Xyleborinus</i> sp 89.06%	7 (maybe 3' mismatch)	some hits going back as both 100% <i>Pseudips mexicanus</i> and <i>Pseudips concinnus</i> . Primer mismatch on reverse sequence is 6bp from the 3' end	
21	17	<i>Ips paracornutus</i>	Not present	Closely related high risk pest	2	0	0	0	0	0	2	NA	0	-		-	-	-	-	-	0	
22	18	<i>Ips plagiographus</i>	Not present	Closely related high risk pest	1	0	0	0	0	0	1	NA	0	-		-	-	-	-	-	0	
23	19	<i>Lymantria ampla</i>	Not present	Closely related high risk pest	1	0	0	0	0	0	1	NA	0	-		-	-	-	-	-	0	
24	20	<i>Lymantria beatrix</i>	Not present	Closely related high risk pest	2	2	2	2	2	2	0	NA	2	92.91-100%	82-95% (including all <i>Lymantria</i> and <i>Orygia</i>)	2 seq	92.91-100%	N	to number of amplicons found w	2	non-specific to <i>Lymantria beatrix</i> , but 2 usable barcodes	
25	21	<i>Lymantria juglandis</i>	Not present	Closely related high risk pest	0	0	0	0	0	0	0	NA	0	-		-	-	-	-	-	0	
26	22	<i>Lymantria mathura</i> subsp. <i>Aurara</i>	Not present	Closely related high risk pest	0	0	0	0	0	0	0	NA	0	-		-	-	-	-	-	0	
27	23	<i>Marchalina hellenica</i>	Not present	Brendan - high risk list	3	0	0	0	0	3	0	NA	0	-		-	-	-	-	-	0	
28	24	<i>Metura elongatus</i>	Not present	Brendan - high risk list	4	0	0	4	4	4	0	1 f 2r	4		NA	4 seq	99.21-100%		100% <i>Metura elongatus</i> 99.21-100%; <i>Eois</i> sp. 88%	2	two on reverse one on first but are central or 5'	
29	25	<i>Orygia papuana</i>	Not present	Closely related high risk pest	5	3	3	3	3	3	2	NA	3	100%		3 seq	100%	Y	100% <i>Orygia papuana</i> 100%; <i>Orygia australis</i> 100%	1		
30	26	<i>Orygia prisca</i>	Not present	Closely related high risk pest	1	0	1	1	1	1	0	5' rev	1	NA		1 seq	100%	Y	100% <i>Orygia prisca</i> ; 94.4% <i>Orygia</i> sp. Unknown	1	mismatch is 5' of reverse primer	
31	27	<i>Orygia thyellina</i>	Not present	FFNZ unwanted pest list	7	0	7	7	7	7	0	3' rev	7	98.4-100%		7 seq	98.4-100% (3bp)	N	99.21-100% <i>Orygia thyellina</i> ; 91.3% <i>Uranophora quadrastigata</i>	4 (maybe 3' mismatch)	it's a maybe if it would work as the mismatches are 6bp 3' of the reverse primer	
32	28	<i>Orygia basalis</i>	Not present	Closely related high risk pest	0	0	0	0	0	0	0		0	-		-	-	-	-	-	0	
33	29	<i>Orygia gonostigma</i>	Not present	Closely related high risk pest	0	0	0	0	0	0	0		0	-		-	-	-	-	-	0	
34	30	<i>Orygia hopkinsi</i>	Not present	Closely related high risk pest	0	0	0	0	0	0	0		0	-		-	-	-	-	-	0	
35	31	<i>Phaulocridium vittatum</i>	Not present	Brendan - high risk list	16	0	0	0	0	0	16	NA	0	-	NA		-	-	-	-	0	
36	32	<i>Platypus semiganosus</i>	Not present	Brendan - high risk list	1	0	1	1	1	1	0	1f 1r	1	NA		1 seq	100%	NA	100% <i>Platypus semiganosus</i> ; 100% <i>Platypus semiganosus</i>	3	1 mismatch each primer but central	
37	33	<i>Neodiprion lecontei</i>	Not present	Biosecurity notifiable organisms order 2016	279	0	1	1	1	1	278	-	1	NA		1 seq	100%	Y	100% <i>Neodiprion lecontei</i> ; 94.44% <i>Neodiprion</i> sp	1		
38	34	<i>Orthotomicus erosus</i>	Not present	Biosecurity notifiable organisms order 2016	7	0	0	0	0	4	3	3 or 4 rev	0	-		-	-	-	-	-	0	
39	35	<i>Pissodes nemorensis</i>	Not present	Biosecurity notifiable organisms order 2016	8	0	3	3	3	3	5	1 rev	3	NA		3 seq	100%	Y	100% <i>Pissodes nemorensis</i> 100%; <i>Pissodes strobi</i> 96.03-100%; <i>Pissodes terminalis</i> 100%	1	Overlap with other <i>Pissodes</i> species in BOLD	
40	36	<i>Melanophila californica</i> (pseudonym)	Not present	Biosecurity notifiable organisms order 2016	0	-	-	-	-	-	0	-	0	-		-	-	-	-	-	0	
41	37	<i>Anoplophora nobilis</i>	Not present	MPI - unwanted, closely related	0	-	-	-	-	-	0	-	0	-		-	-	-	-	-	0	
42	38	<i>Anoplophora malasiaca</i>	Not present	MPI - unwanted, closely related	1	1	1	1	1	1	0		1	NA		1 seq	-	-	-	-	0	Same barcode as <i>altis</i> for <i>Anoplophora chinensis</i>
43	39	<i>Anoplophora horsfieldi</i>	Not present	EPPO alert list 2025	3	3	3	3	3	3	0		3	92.13-100%		3 seq	92.13-100%	N	100% <i>Anoplophora horsfieldi</i> (x1), <i>Dorcadion</i> sp 95.28%, <i>Anoplophora</i> sp 95.28% <i>Dorcadion</i> sp, 99.21-100%	2	BOLD on had hits for spelling <i>horsfieldi</i> . Not really very specific to species level, lots of 100% hits to other <i>Anoplophora</i> , and the barcodes seem not very specific in bold either. One removed that was closer to <i>glabripennis</i>	
44																						
45			Within family specificity check										Accuracy		Specificity							
46	All remaining barcodes		Status	Source	Sequences*	0	1	2	3	4	no amp found	Mismatch location	Usable barcodes ? [†]	% ident in species	% ident in family	Mega alignment	Seq ID	Consensus?	BOLD search	nBLAST	final number of barcodes for list	notes for specificity of COI/ primer mismatches
47	40	<i>Cinara atlantica</i>	Not present	Brendan - high risk list	14	0	12	12	12	12	2		12	100%		12 seq	100%	Y	100% <i>Cinara atlantica</i> and <i>Cinara melajana</i>	1		
48	41	<i>Cinara pinivora</i>	Not present	Brendan - high risk list	61	0	57	60	60	60	1		57	97.64-100%	90-94.5%	57 seq	97.64-100%	Y	97.63-100% <i>Cinara pinivora</i> ; 97.64 <i>Cinara terminalis</i>	3	11 <100% sequence ID.	
49	42	<i>Cinara cupressi</i>	Not present	FFNZ unwanted pest list	44	0	0	43	43	43	1		43	99.21-100%		43 seq	99.21-100%	N	99.21-100% <i>Cinara cupressi</i> , <i>Cinara</i> spp, 95.28% <i>Cinara tujaefolia</i>	3		
50	43	<i>Coptotermes acinaciformis</i>	Not present	Brendan - high risk list	91	0	52	77	85	85	6		85	95.28-100%		85 seq	95.28-100%	N	96.85-100% <i>Coptotermes acinaciformis</i> ; 96.06% <i>Coptotermes michaelsoni</i>	12		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
51	44	<i>Ecotropis excursaria</i>	Not present	Brendan - high risk list	73	72	72	72	72	73	0	NA	72	96.85-100%	NA	72 seq	96.85-100%	N	99.21-100% <i>Ecotropis excursaria</i>	96.85% <i>Ecotropis excursaria</i> ; 96.05% <i>Cosciniophyta improbana</i>	7	
52	45	<i>Epiphyas caryotis</i>	Not present	Brendan - high risk list	24	24	24	24	24	24	0	NA	24	98.43-100%		24 seq	98.43-100%	N	99.21-100% <i>Epiphyas caryotis</i> , 98.43% <i>Epiphyas fabricata</i>	99.21-100% <i>Epiphyas caryotis</i> , 98.43% <i>Epiphyas fabricata</i>	3	
53	46	<i>Eulachnus agilis</i>	Not present	Brendan - high risk list	101	0	0	0	101	101	0	1f, 2r	101	96.06-100%		101 seq	96.06-100%	N	96.85-100% <i>Eulachnus</i> sp., 96.85% <i>Eulachnus agilis</i>	99.21-100% <i>Eulachnus agilis</i> , 96.85% <i>Eulachnus rileyi</i>	4	Three mismatches overall. 1 on f 32 on r.
54	47	<i>Hylastes opacus</i>	Not present	Brendan - high risk list	38	0	0	13	31	31	7	1f, 1 or 2r	31	96.06-100%		31 seq	96.06-100%	N	97.64-100% <i>Hylastes opacus</i> , 89.76% <i>Trigonophorus</i> sp.		7	
55	48	<i>Hyurgus ligniperda</i>	established - high risk	Brendan - high risk list	33	29	29	29	29	29	4	NA	29	96.85-100%		29 seq	96.85-100%	N	97.64-100% <i>Hyurgus ligniperda</i> , 89.69% <i>Sitona striatellus</i>	96.85-100% <i>Hyurgus ligniperda</i> , 89.69% <i>Sitona striatellus</i>	4	
56	49	<i>Leptoglossus occidentalis</i>	Not present	Brendan - high risk list	108	52	52	52	52	52	56	NA	52	99.21-100%	82-95% (including all <i>Lymantria</i> and <i>Orygia</i>)	52 seq	99.21-100%	N	99.21-100% <i>Leptoglossus occidentalis</i> , 93.65-95.24% <i>leptoglossus clypealis</i>	98.41-100% <i>Leptoglossus occidentalis</i> , 93.65-95.24% <i>leptoglossus clypealis</i>	5	
57	50	<i>Lymantria dispar</i>	Not present	Brendan - high risk list	3599	3077	3241	3267	3288	3288	311		3274	87.1-100%		3274 seq	87.1-100%	N	99.21-100% <i>Lymantria dispar</i>	99.21-100% <i>Lymantria dispar</i>	146	top barcode was 1,789 of ~3000 seq. dominant barcode
58	51	<i>Lymantria monacha</i>	Not present	Brendan - high risk list	396	313	321	325	326	327	69		329	86.61-100%		329 seq	86.61-100%	N	99.21-100% <i>Lymantria monacha</i>	98.43-100% <i>Lymantria monacha</i>	28	removed a few that BOLD couldn't resolve the barcode to species level
59	52	<i>Lymantria subsp dispar asiatica</i>	Not present	Brendan - high risk list	92	79	79	79	79	79	13	NA	79	99.21-100%		79 seq	99.21-100%	N	100% <i>Lymantria dispar</i>	99.21-100% <i>Lymantria dispar</i>	5	
60	53	<i>Lymantria subsp dispar japonica</i>	Not present	Brendan - high risk list	68	56	56	56	56	56	12	NA	56	99.21-100%		56 seq	99.21-100%	N	100% <i>Lymantria dispar</i>	99.21-100% <i>Lymantria dispar</i>	4	
61	54	<i>Lymantria subsp. Dispar dispar</i>	Not present	Brendan - high risk list	390	375	376	376	376	376	14		376	100%		376 seq	100%	N	100% <i>Lymantria dispar</i>	99.21-100% <i>Lymantria dispar</i>	8	
62	55	<i>Teia anartoides</i>	Not present	Closely related high risk pest	21	0	0	20	20	20	1		19	99.21-100%	82-95% (including all <i>Lymantria</i> and <i>Orygia</i>)	19 seq	99.21-100%	N	99.21-100% <i>Teia anartoides</i> , 96.63-97.62% <i>Teia athlophora</i>	99.21-100% <i>Teia anartoides</i> , 96.63-97.62% <i>Teia athlophora</i>	2	2 rev primer mismatches
63	56	<i>Lymantria mathura</i>	Not present	Closely related high risk pest	87	47	83	87	87	87	0	1f or 1r	86	88.98-100%		86 seq	88.98-100%	N	99.21-100% <i>Lymantria math</i> , 92.91% <i>Ophithalmis albosignaria</i>	95.28-100% <i>Lymantria mathura</i> , 92.91% <i>Ophithalmis albosignaria</i>	11	
64	57	<i>Lymantria obfuscata</i>	Not present	Closely related high risk pest	14	1	12	13	14	14	0	0/1 on f and r	13	96.06-100%		13 seq	96.06-100%	N	96.06-100% <i>Lymantria obfuscata</i> , 95.28% <i>Myrmecopsis strigosa</i> , 96.24% <i>Lymantria dispar</i>	96.06-100% <i>Lymantria obfuscata</i> , 95.24% <i>Lymantria dispar</i>	5	Removed a barcode that was only classified to the Order level. 95% match to two other species
65	58	<i>Lymantria xyliina</i>	Not present	Closely related high risk pest	74	69	69	69	69	69	5	NA	69	98.43-100%		69 seq	98.43-100%	N	99.21-100% <i>Lymantria xyliina</i> , 99.21-100% <i>Lymantria schaeferi</i>	99.21-100% <i>Lymantria xyliina</i> , 99.21-100% <i>Lymantria schaeferi</i>	7	
66	59	<i>Orygia leucostigma</i>	Not present	Closely related high risk pest	152	0	150	151	151	151	1		150	97.64-100%		150 seq	97.64-100%	N	99.21-100% <i>Orygia leucostigma</i> , 100% <i>Orygia leucostigma</i>	99.21-100% <i>Orygia leucostigma</i> , 100% <i>Orygia leucostigma</i>	9	No species overlaps in BOLD database
67	60	<i>Orygia postica</i>	Not present	Closely related high risk pest	14	11	14	14	14	14	0		13	88.98-100%	NA	13 seq	88.98-100%	N	99.21-100% <i>Orygia postica</i> , 99.21% <i>Orygia australis</i>	99.21-100% <i>Orygia postica</i> , 99.21% <i>Orygia australis</i>	6	Some overlap with <i>Orygia australis</i>
68	61	<i>Orygia antiqua</i>	Not present	Brendan - high risk list	124	0	120	121	121	121	3		120	88.98-100%		120 seq	88.98-100%	N	99.21-100% <i>Orygia antiqua</i> , 93.7% <i>Prays eleae</i>	96.06-100% <i>Orygia antiqua</i> , 93.7% <i>Prays eleae</i>	5	
69	62	<i>Orygia australis</i>	Not present	Brendan - high risk list	71	70	70	70	70	70	1	NA	70	94.49-100%		70 seq	94.49-100%	N	99.21-100% <i>Orygia australis</i> , 99.21% <i>Orygia papuana</i>	96.06-100% <i>Orygia australis</i> , 96.85% <i>Orygia postica</i>	8	Some overlap with <i>Orygia postica</i>
70	63	<i>Monochamus alternatus</i>	Not present	Brendan - high risk list	105	0	39	39	39	39	66	rev middle	39	98.43-100%		39 seq	98.43-100%	N	99.21-100% <i>monochamus alternatus</i>	99.21-100% <i>monochamus alternatus</i>	7	
71	64	<i>Nysius vinitor</i>	Not present	Brendan - high risk list	15	4	15	15	15	15	0		15	97.64-100%		15 seq	97.64-100%	N	99.21-100% <i>Nysius vinitor</i> , 99.21% <i>Nysius plebius</i>	99.21-100% <i>Nysius vinitor</i> , 99.21% <i>Nysius plebius</i>	6	Seems to be some taxonomic mixing with <i>Nysius vinitor</i> and <i>Nysius caledoniae</i>
72	65	<i>Paropsis charybdis</i>	established - high risk	Brendan - high risk list	32	0	0	0	29	29	3		29	97.64-100%	NA	29 seq	97.64-100%	N	98.43-100% <i>Paropsis charybdis</i> , 88.1-88.89% <i>Gonioctena pallida</i>	98.43-100% <i>Paropsis charybdis</i> , 88.1-88.89% <i>Gonioctena pallida</i>	5	
73	66	<i>Pineus strobi</i>	Not present	Brendan - high risk list	73	0	0	4	70	71	2	1f, 1-2 r	70	92.86-100%		70 seq	92.86-100%	N	98.43-100% <i>Pineus strobi</i> , 94.44% <i>Pineus pini</i> , 99.21% <i>Pineus pini</i>	98.43-100% <i>Pineus strobi</i> , 94.44% <i>Pineus pini</i> , 99.21% <i>Pineus pini</i>	8	Some overlap with <i>Pineus</i> species
74	67	<i>Rhagium inquisitor</i>	Not present	Brendan - high risk list	149	2	76	79	79	80	69	0-1f, 0-2 r	80	90.55-100%		80 seq	90.55-100%	N	96.03-100% <i>Rhagium inquisitor</i>	96.03-100% <i>Rhagium inquisitor</i> , 94.49% <i>Syntomium aenum</i>	11	
75	68	<i>Sirex noctilio</i>	established - high risk	Brendan - high risk list	73	0	73	73	73	73	0	rev middle	73	98.43-100%		73 seq	98.43-100%	N	98.43-100% <i>Sirex noctilio</i> , 92.06% <i>Sirex nitobei</i>	98.43-100% <i>Sirex noctilio</i> , 92.06% <i>Sirex nitobei</i>	3	
76	69	<i>Urocerus gigas</i>	Not present	Brendan - high risk list	21	0	0	0	20	20	1	1f, 2r	20	97.64-100%	lps: 79-98%	20 seq	97.64-100%	N	97.64-100% <i>Urocerus gigas</i> , 94.49-95.28% <i>Urocerus flavicornis</i> , 96.06% <i>Urocerus augur</i>	99.21-100% <i>Urocerus gigas</i> , 94.49% <i>Urocerus flavicornis</i> , 96.06% <i>Urocerus augur</i>	3	<i>Urocerus flavicornis</i> a subspecies of <i>Urocerus gigas</i> ?
77	70	<i>Ips pini</i>	Not present	Closely related high risk pest	16	5	12	12	12	12	4		8	78.52-100%		8 seq	78.52-100%	N	98.41-100% <i>Ips pini</i> , 99.21% <i>Ips perturbatus</i>	98.41-100% <i>Ips pini</i> , 99.21% <i>Ips perturbatus</i>	5	Low barcode similarities, but BOLD has alt3 and alt2 to species level
78	71	<i>Ips sexdentatus</i>	Not present	Closely related high risk pest	93	69	72	75	76	76	17		75	94.49-100%		75 seq	94.49-100%	N	99.21-100% <i>Ips sexdentatus</i> , 88.98% <i>Phyllotreta pyri</i>	95.28-100% <i>Ips sexdentatus</i> , 88.98% <i>Phyllotreta pyri</i>	21	
79	72	<i>Ips typographus</i>	Not present	Closely related high risk pest	223	70	71	0	0	0	223		71	97.64-100%		71 seq	97.64-100%	N	100% <i>Ips typographus</i>	100% <i>Ips typographus</i> , 98.43% <i>Dendroctera collaris</i> (x1), 98.43% <i>Ips cembrae</i>	9	
80	73	<i>Ips cembrae</i>	Not present	Closely related high risk pest	11	1	1	1	1	1	10		1	100%		1 seq	100%	Y	100% <i>Ips typographus</i>	100% <i>Ips typographus</i> <i>Ips cembrae</i>	1	Seems inter mixed with <i>Ips typographus</i> - exactly the same barcode as <i>Ips typographus</i> COI alt2
81	74	<i>Anoplophora glabripennis</i>	Not present	FFNZ unwanted pest list	1048	1031	1037	1037	1037	1037	11		1037	98.43-100%	NA	1037 seq	98.43-100%	Y	100% <i>Anoplophora glabripennis</i>	100% <i>Anoplophora glabripennis</i>	11	
82	75	<i>Anoplophora chinensis</i>	Not present	Closely related pest species	55	42	44	44	44	44	11	1f	44	96.83-100%		44 seq	96.83-100%	N	97.64-100% <i>Anoplophora chinensis</i> , 96.85-98.43% <i>Anoplophora macularia</i> , 97.64-99.21% <i>Anoplophora macularia</i>	99.21-100% <i>Anoplophora chinensis</i> , 97.64-99.21% <i>Anoplophora macularia</i>	10	Removed one that closest species was <i>Anoplophora macularia</i> . Seem quite overlapping at species level
83	76	<i>Anoplophora macularia</i>	Not present	MPI - unwanted, closely related	31	23	23	23	23	23	8	-	23	95.28-100%		23 seq	95.28-100%	N	100% <i>Anoplophora macularia</i> , 97.64-98.43% <i>Anoplophora chinensis</i>	98.43-100% <i>Anoplophora macularia</i> , 97.64-98.43% <i>Anoplophora chinensis</i>	9	Two barcodes said chinensis so were removed. Only alt2 and alt3 were to species level. As with others, some overlap
84	77	<i>Halymorphia halys</i>	Not present	FFNZ unwanted pest list	3141	2055	2064	2065	2065	2065	1076		2064	85-100%		2064 seq	85-100%	N	99.21-100% <i>Halymorphia halys</i>	100% <i>Halymorphia halys</i>	75	Removed one barcode as was Genus Halys
85	78	<i>Cladus grandis</i>	Present	FFNZ unwanted pest list	13	13	13	13	13	13	0		13	97.64-100%	NA	13 seq	97.64-100%	N	98.43-100% <i>Cladus grandis</i> , 94.49% <i>Cladus sp</i>	98.43-100% <i>Cladus grandis</i> , 94.49% <i>Cladus sp</i>	4	
86	79	<i>Xylosandrus crassiusculus</i>	Not present	FFNZ unwanted pest list	831	1	47	723	727	791	40		724	92.13-100%		724 seq	92.13-100%	N	96.85-100% <i>Xylosandrus crassiusculus</i> , 91.34% <i>Xyleborus bolivianus</i>	96.85-100% <i>Xylosandrus crassiusculus</i> , 91.34% <i>Xyleborus bolivianus</i>	22	
87	80	<i>Cryptotermes brevis</i>	Not present	FFNZ unwanted pest list	13	0	0	11	13	13	0		12	96.06-100%		12 seq	96.06-100%	N	95.28-100% <i>Cryptotermes brevis</i> , 90.48-92.06% <i>Odontotermes formosus</i>	96.85-100% <i>Cryptotermes brevis</i> , 90.48-92.06% <i>Odontotermes formosus</i>	4	
88	81	<i>Thaumetopoea pityocampa</i>	Not present	FFNZ unwanted pest list	223	0	67	84	84	84	139		84	81.1-100%		84 seq	81.1-100%	N	97.64-100% <i>Thaumetopoea pityocampa</i> , 94.44% <i>Oxytenis gigantea</i>	97.64-100% <i>Thaumetopoea pityocampa</i> , 94.44% <i>Oxytenis gigantea</i>	13	

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	
89	82	<i>Corythucha ciliata</i>	Not present	FFNZ unwanted pest list	85	0	72	73	73	73	12		73	96.06-100%	NA	73 seq	96.06-100%	N	96.06-100% <i>Corythucha ciliata</i> , 96.06% <i>Trigloea</i> sp. 95.28% <i>Corythucha</i> sp.	96.06-100% <i>cCorythucha ciliata</i> , 96.06% <i>Trigloea</i> sp. 95.28% <i>Corythucha</i> sp.	9		
90	83	<i>Rhyacionia buoliana</i>	Not present	FFNZ unwanted pest list	44	42	42	42	42	42	2		42	97.64-100%		42 seq	97.64-100%	N	99.21-100% <i>Rhyacionia buoliana</i> , 96.85% <i>Polia purpurissata</i>	99.21-100% <i>Rhyacionia buoliana</i> , 96.85% <i>Polia purpurissata</i>	5	removed one barcode as it matches more with another genus	
91	84	<i>Xanthogaleruca luteola</i>	Not present	FFNZ unwanted pest list	17	0	0	17	17	17	0		17	98.43-100%		17 seq	98.43-100%	N	99.21-100% <i>Xanthogaleruca luteola</i> , 89.68% <i>Sonoma brassotomensis</i>	99.21-100% <i>Xanthogaleruca luteola</i> , 89.68% <i>Sonoma brassotomensis</i>	4		
92	85	<i>Euxwallacea fornicatus</i>	Not present	FFNZ unwanted pest list	180	3	54	132	144	144	36		147	84-100%		147 seq	84-100%	N	94.44-100% <i>Euxwallacea fornicatus</i> , 93.7-100% <i>Euxwallacea kuroshio</i>	93.7-100% <i>Euxwallacea fornicatus</i>	54	Some overlap with <i>Euxwallacea kuroshio</i>	
93	86	<i>Monochamus alternatus</i>	Not present	Brendan - high risk list	105	0	39	39	39	39	66	1 rev	39	97.64-100%		39 seq	97.64-100%	N	98.43-100% <i>Monochamus alternatus</i>	99.21-100% <i>monochamus alternatus</i>	8		
94	87	<i>Hyphantria cunea</i>	Not present	FFNZ unwanted pest list	493	381	426	426	428	428	65		425	91.27-100%		425 seq	91.27-100%	N	97.64-100% <i>Hyphantria cunea</i> <i>Anthrenus sinensis</i> (x1) <i>Anthrenus picturatus hintoni</i> (x1)	100% <i>Hyphantria cunea</i> <i>Anthrenus sinensis</i> (x1) <i>Anthrenus picturatus hintoni</i> (x1)	24	single overlaps with 2 species, possibly ID errors?	
95	88	<i>Hylobius abietis</i>	Not present	FFNZ unwanted pest list	57	0	0	56	56	56	1		56	98.43-100%		56 seq	98.43-100%	N	99.21-100% <i>Hylobius abietis</i> <i>transversovittatus</i> (x1)	<i>Hylobius abietis</i> 99.21-100%, <i>Scepticus uniformis</i> 88.98%	6	One barcode came up with 100% confidence of <i>Cryptocephalus nitidus</i> , was removed	
96	89	<i>Arhopalus rusticus</i>	Not present	Brendan - high risk list	68	54	64	65	65	65	3		64	82.68-100%		64 seq	82.68-100%	N	97-100% <i>Anthrophalus rusticus</i> , <i>arhopalus</i> sp. 98.43% <i>tetropium castaneum</i>	95.28-100% <i>Arhopalus rusticus</i> , <i>arhopalus</i> sp. 98.43% <i>tetropium castaneum</i>	33	A lot of unique sequences for 64 barcodes. Removed one as was linked to another genus	
97	90	<i>Buprestis aurulentula</i>	Not present	Biosecurity notifiable organisms order 2016	10	9	10	10	10	10	0	1 rev	10	97.64-100%		10 seq	97.64-100%	N	97.64-100% <i>Buprestis aurulentula</i>	89.76% <i>Psychodopgys amazonensis</i> , 89.59% <i>Enimus minus</i>	5		
98	91	<i>Coptotermes formosanus</i>	Not present	Biosecurity notifiable organisms order 2016	96	0	0	70	70	70	26	2 rev	70	96.06-100%		70 seq	96.06-100%	N	96.06-100% <i>Coptotermes formosanus</i> , 95.28% <i>Coptotermes elisae</i> , 97.64% <i>Coptotermes acinaciformis</i>	96.06-100% <i>Coptotermes formosanus</i> , 96.06% <i>Coptotermes acinaciformis</i> , 96.06% <i>Coptotermes curvignathus</i>	2		
99	92	<i>Dendroctonus valens</i>	Not present	Biosecurity notifiable organisms order 2016	181	0	2	15	16	16	165	rev: 1 or 2	16	82.68-100%		16 seq	82.68-100%	N	96.85-100% <i>Dendroctonus valens</i> , 82.68% <i>Cneumonys vismiacolems</i>	96.06-100% <i>Dendroctonus valens</i> , 82.68% <i>Cneumonys vismiacolems</i>	11		
100	93	<i>Monochamus</i> spp	Not present	Biosecurity notifiable organisms order 2016	637	197	317	325	327	327	310	-	-	78.74-100%		310 seq	78.74-100%	N	99.21-100% <i>Monochamus</i> sp.	99.21-100% <i>Monochamus</i> sp.	69	Did a BOLD search for the whole genus. Removed one that was <i>Anophophora glabripennis</i> , <i>Trigloea apiaris</i> , <i>Aegsoma scabricorne</i> and one to just family level	
101	94	<i>Tomicus piniperda</i>	Not present	Biosecurity notifiable organisms order 2016	159	0	0	36	36	36	123	2 rev 3'	0	-					-	-	0	Reverse mismatches were in the first 5bp of the 3' end of the primer	
102	95	<i>Euzophera semifuneralis</i>	Not present	EPPO alert list 2025	29	28	29	29	29	29	0		29	94.49-100%		29 seq	94.49-100%	N	95.28-100% <i>Euzosphera semifuneralis</i> , 95.28-100% <i>Euzosphera habrella</i>	100% <i>Euzosphera semifuneralis</i> , 100% <i>Euzosphera habrella</i> , 95.28% <i>Euzosphera</i> sp.	4	Not very species specific	
103	96	<i>Monema flavescens</i>	Not present	EPPO alert list 2025	81	76	77	77	77	77	4		76	92.91-100%		76 seq	92.91-100%	N	97.64-100% <i>monema flavescens</i> , 97.64-99.21% <i>Monema rubriceps</i>	97.64-100% <i>Monema flavescens</i> , 97.64% <i>Monema rubriceps</i>	17	Slight overlap with <i>rubriceps</i> , though not at the highest similarity level	
104	97	<i>Trachymela sloanei</i>	Present	EPPO alert list 2025	21	1	1	1	1	1	20		1	NA		1 seq	NA	Y	100% <i>Trachymela</i> sp	100% <i>Trachymela</i> sp. 98.98% <i>Paropsis maculata</i>	1	ID to genus level in BOLD	
105	* Seq from BOLD sometimes include other barcodes i.e 18S/12S																						
106	Usable barcode takes into account the location of the mismatches. i.e if too close to 3' end (<10bp) not usable. And if more than 2 on a primer not usable																						

[illegible]

	A	B	C	D	E	F	G	H	I	J	K
38											
39		All remaining barcodes	Status	Source	Sequences*	0	1	2	3	4	no amp found
40	33	<i>Anoplophora glabripennis</i>	Not present	FFNZ unwanted pest list	1048	703	703	703	703	703	345
41	34	<i>Arhopalus rusticus</i>	Not present	Brendan - high risk list	68	3	3	3	3	3	65
42	35	<i>Buprestis aurulenta</i>	Not present	Biosecurity notifiable organisms order 2016	10	0	0	0	0	0	10
43	36	<i>Cinara atlantica</i>	Not present	Brendan - high risk list	14	0	0	0	0	0	14
44	37	<i>Cinara cupressi</i>	Not present	FFNZ unwanted pest list	44	0	0	0	0	0	44
45	38	<i>Cinara pinivora</i>	Not present	Brendan - high risk list	61	0	0	0	0	0	61
46	39	<i>Cladius grandis</i>	Present	FFNZ unwanted pest list	13	1	1	1	1	1	12
47	40	<i>Coptotermes acinaciformis</i>	Not present	Brendan - high risk list	91	76	77	77	77	77	14
48	41	<i>Coptotermes formosanus</i>	Not present	Biosecurity notifiable organisms order 2016	96	8	19	19	19	19	77
49	42	<i>Corythucha ciliata</i>	Not present	FFNZ unwanted pest list	85	2	2	2	2	2	83
50	43	<i>Cryptotermes brevis</i>	Not present	FFNZ unwanted pest list	13	1	1	1	1	1	12
51	44	<i>Dendroctonus valens</i>	Not present	Biosecurity notifiable organisms order 2016	181	11	11	11	11	11	170
52	45	<i>Ectropis excursaria</i>	Not present	Brendan - high risk list	73	0	0	0	0	0	73
53	46	<i>Epiphyas caryotis</i>	Not present	Brendan - high risk list	24	0	0	0	0	0	24
54	47	<i>Eulachnus agilis</i>	Not present	Brendan - high risk list	101	0	0	0	0	0	101
55	48	<i>Euwallacea fornicatus</i>	Not present	FFNZ unwanted pest list	180	24	24	24	24	24	156
56	49	<i>Euzophera semifuneralis</i>	Not present	EPPO alert list 2025	29	1	1	1	1	1	28
57	50	<i>Halyomorpha halys</i>	Not present	FFNZ unwanted pest list	3141	143	143	143	143	143	2998
58	51	<i>Hylastes opacus</i>	Not present	Brendan - high risk list	38	4	18	18	18	18	20
59	52	<i>Hylobius abietis</i>	Not present	FFNZ unwanted pest list	57	0	1	1	1	1	56
60	53	<i>Hylurgus ligniperda</i>	established - high risk	Brendan - high risk list	33	20	20	20	20	20	4
61	54	<i>Hyphantria cunea</i>	Not present	FFNZ unwanted pest list	493	41	49	49	49	49	444
62	55	<i>Ips cembrae</i>	Not present	Closely related high risk pest	11	0	0	0	0	0	11
63	56	<i>Ips pini</i>	Not present	Closely related high risk pest	16	1	1	1	1	1	15
64	57	<i>Ips sexdentatus</i>	Not present	Closely related high risk pest	93	17	17	17	17	17	76
65	58	<i>Ips typographus</i>	Not present	Closely related high risk pest	223	3	39	39	39	39	184
66	59	<i>Leptoglossus occidentalis</i>	Not present	Brendan - high risk list	108	3	3	3	3	3	105
67	60	<i>Lymantria dispar</i>	Not present	Brendan - high risk list	3599	195	196	196	197	200	3399
68	61	<i>Lymantria mathura</i>	Not present	Closely related high risk pest	87	1	1	1	1	1	86
69	62	<i>Lymantria monacha</i>	Not present	Brendan - high risk list	396	0	0	0	0	0	396
70	63	<i>Lymantria obfuscata</i>	Not present	Closely related high risk pest	14	0	0	0	0	0	14
71	64	<i>Lymantria subsp dispar asiatica</i>	Not present	Brendan - high risk list	92						92

	A	B	C	D	E	F	G	H	I	J	K
72	65	<i>Lymantria subsp dispar japonica</i>	Not present	Brendan - high risk list	68						68
73	66	<i>Lymantria subsp. Dispar dispar</i>	Not present	Brendan - high risk list	390						390
74	67	<i>Lymantria xyliana</i>	Not present	Closely related high risk pest	74	0	0	0	0	0	74
75	68	<i>Monema flavescens</i>	Not present	EPPO alert list 2025	81	3	3	3	8	13	68
76	69	<i>Monochamus alternatus</i>	Not present	Brendan - high risk list	105	5	5	5	5	5	100
77	70	<i>Monochamus</i> spp	Not present	Biosecurity notifiable organisms order 2016	637						637
78	71	<i>Nysius vinitor</i>	Not present	Brendan - high risk list	15	0	0	0	0	0	15
79	72	<i>Orgyia antiqua</i>	Not present	Brendan - high risk list	124	0	0	0	0	0	124
80	73	<i>Orgyia australis</i>	Not present	Brendan - high risk list	71	0	0	0	0	0	71
81	74	<i>Orgyia leucostigma</i>	Not present	Closely related high risk pest	152	0	0	0	0	0	152
82	75	<i>Orgyia postica</i>	Not present	Closely related high risk pest	14	0	0	0	0	0	14
83	76	<i>Paropsis charybdis</i>	established - high risk	Brendan - high risk list	32	0	0	0	0	0	32
84	77	<i>Pineus strobi</i>	Not present	Brendan - high risk list	73	0	0	0	0	0	73
85	78	<i>Rhagium inquisitor</i>	Not present	Brendan - high risk list	149	0	0	0	0	0	149
86	79	<i>Rhyacionia buoliana</i>	Not present	FFNZ unwanted pest list	44	0	0	0	0	0	44
87	80	<i>Sirex noctilio</i>	established - high risk	Brendan - high risk list	73	0	14	14	14	14	59
88	81	<i>Teia anartoides</i>	Not present	Closely related high risk pest	21	0	0	0	0	0	21
89	82	<i>Thaumetopoea pityocampa</i>	Not present	FFNZ unwanted pest list	223	0	1	1	1	1	222
90	83	<i>Tomicus piniperda</i>	Not present	Biosecurity notifiable organisms order 2016	159	12	15	15	15	15	144
91	84	<i>Trachymela sloanei</i>	Present	EPPO alert list 2025	21	0	0	0	0	0	21
92	85	<i>Urocerus gigas</i>	Not present	Brendan - high risk list	21	2	2	2	2	2	19
93	86	<i>Xanthogaleruca luteola</i>	Not present	FFNZ unwanted pest list	17						17
94	87	<i>Xylosandrus crassiusculus</i>	Not present	FFNZ unwanted pest list	831	3	7	7	7	7	824
95	* Seq from BOLD sometimes include other barcodes i.e 18S/12S										
96	¹ usable barcode takes into account the location of the mismatches. I.e if too close to 3; end (<10bp) not usable. And if more than 2 on a primer not usable										

	A	B	C	D	E	F	G	H	I	J
1	Table S3	Computational PCR results using the aquatic invertebrate COI primers from Wilderlab, set to strict mismatch threshold of one mismatch per primer (2 in total)								
2	#	Species	Status	Source	Sequences*	0	1	2	no amp found	Mismatch location
3	1	<i>Agrotis radians</i>	Not present	Brendan - high risk list	5	4	0		1	NA
4	2	<i>Anoplophora horsfieldii</i>	Not present	EPPO alert list 2025	3	3	0		0	-
5	3	<i>Anoplophora malasiaca</i>	Not present	MPI - unwanted, closely related	1	1	0		0	
6	4	<i>Heterobostrychus aequalis</i>	Not present	FFNZ unwanted pest list	5	0	3		2	reverse primer
7	5	<i>Hylastes ater</i>	established - high risk	Brendan - high risk list	5	1	4		0	3'-middle; rev
8	6	<i>Hylastes nigrinus</i>	Not present	Closely related high risk pest	5	0	4		1	5' rev
9	7	<i>Hypothenemus eruditus</i>	Not present	Brendan - high risk list	15	1	0	4	10	5' rev; 5' for
10	8	<i>Ips grandicollis</i>	Not present	Brendan - high risk list	11	1	4		6	3' rev
11	9	<i>Lymantria beatrix</i>	Not present	Closely related high risk pest	2	2	0		0	NA
12	10	<i>Neodiprion lecontei</i>	Not present	Biosecurity notifiable organisms order 2016	279	0	1		278	-
13	11	<i>Orgyia papuana</i>	Not present	Closely related high risk pest	5	3	0		2	NA
14	12	<i>Orgyia prisca</i>	Not present	Closely related high risk pest	1	0	1		0	5' rev
15	13	<i>Orgyia thyellina</i>	Not present	FFNZ unwanted pest list	7	0	6	1	0	3' rev
16	14	<i>Pissodes nemorensis</i>	Not present	Biosecurity notifiable organisms order 2016	8	0	3		5	1 rev
17	15	<i>Platypus semigranosus</i>	Not present	Brendan - high risk list	1	0	0	1	0	1f 1r
18	16	<i>Pseudips mexicanus</i>	Not present	Closely related high risk pest	29	0	0	5	24	3' rev
19	All remaining barcodes		Status	Source	Sequences*	0	1	2	no amp found	Mismatch location
20	17	<i>Anoplophora chinensis</i>	Not present	Closely related pest species	55	42	2		11	1f
21	18	<i>Anoplophora glabripennis</i>	Not present	FFNZ unwanted pest list	1048	1031	6		11	
22	19	<i>Anoplophora macularia</i>	Not present	MPI - unwanted, closely related	31	23	0		8	-
23	20	<i>Arhopalus rusticus</i>	Not present	Brendan - high risk list	68	54	10		4	
24	21	<i>Buprestis aurulenta</i>	Not present	Biosecurity notifiable organisms order 2016	10	9	1		0	1 rev
25	22	<i>Cinara atlantica</i>	Not present	Brendan - high risk list	14	0	0	12	2	1f 1r
26	23	<i>Cinara cupressi</i>	Not present	FFNZ unwanted pest list	44	0	0	57	-13	1f 1r
27	24	<i>Cinara pinivora</i>	Not present	Brendan - high risk list	61	0	0	43	18	1f 1r

	A	B	C	D	E	F	G	H	I	J
28	25	<i>Cladius grandis</i>	Present	FFNZ unwanted pest list	13	13	0		0	
29	26	<i>Coptotermes acinaciformis</i>	Not present	Brendan - high risk list	91	0	52	1	38	
30	27	<i>Corythucha ciliata</i>	Not present	FFNZ unwanted pest list	85	0	72	1	12	
31	28	<i>Dendroctonus valens</i>	Not present	Biosecurity notifiable organisms order 2016	181	0	2		179	rev; 1 or 2
32	29	<i>Ectropis excursaria</i>	Not present	Brendan - high risk list	73	72	0		1	NA
33	30	<i>Epiphyas caryotis</i>	Not present	Brendan - high risk list	24	24	0		0	NA
34	31	<i>Euwallacea fornicatus</i>	Not present	FFNZ unwanted pest list	180	3	51		126	
35	32	<i>Euzophera semifuneralis</i>	Not present	EPPO alert list 2025	29	28	1		0	
36	33	<i>Halyomorpha halys</i>	Not present	FFNZ unwanted pest list	3141	2055	9		1077	
37	34	<i>Hylastes opacus</i>	Not present	Brendan - high risk list	38	0	0	9	29	1f, 1 or 2r
38	35	<i>Hylobius abietis</i>	Not present	FFNZ unwanted pest list	57	0	0	55	2	1f 1r
39	36	<i>Hylurgus ligniperda</i>	established - high risk	Brendan - high risk list	33	0	29		4	NA
40	37	<i>Hyphantria cunea</i>	Not present	FFNZ unwanted pest list	493	381	45		67	
41	38	<i>Ips cembrae</i>	Not present	Closely related high risk pest	11	1	0		10	
42	39	<i>Ips pini</i>	Not present	Closely related high risk pest	16	5	7		4	
43	40	<i>Ips sexdentatus</i>	Not present	Closely related high risk pest	93	69	3	1	20	
44	41	<i>Ips typographus</i>	Not present	Closely related high risk pest	223	70	1		152	
45	42	<i>Leptoglossus occidentalis</i>	Not present	Brendan - high risk list	108	52	0		56	NA
46	43	<i>Lymantria dispar</i>	Not present	Brendan - high risk list	3599	3077	164		358	1f
47	44	<i>Lymantria mathura</i>	Not present	Closely related high risk pest	87	47	36	4	0	1f or 1r
48	45	<i>Lymantria monacha</i>	Not present	Brendan - high risk list	396	313	11		72	1f
49	46	<i>Lymantria obfuscata</i>	Not present	Closely related high risk pest	14	1	11	1	1	0/1 on f and r
50	47	<i>Lymantria subsp dispar asiatica</i>	Not present	Brendan - high risk list	92				92	NA
51	48	<i>Lymantria subsp dispar japonica</i>	Not present	Brendan - high risk list	68				68	NA
52	49	<i>Lymantria subsp. Dispar dispar</i>	Not present	Brendan - high risk list	390				390	
53	50	<i>Lymantria xyliana</i>	Not present	Closely related high risk pest	74	68	1		5	NA
54	51	<i>Monema flavescens</i>	Not present	EPPO alert list 2025	81	76	1		4	
55	52	<i>Monochamus alternatus</i>	Not present	Brendan - high risk list	105	0	39		66	rev middle
56	53	<i>Monochamus alternatus</i>	Not present	Brendan - high risk list	105	0	39		66	1 rev
57	54	<i>Monochamus spp</i>	Not present	Biosecurity notifiable organisms order 2016	637	23	37	3	574	-
58	55	<i>Nysius vinitor</i>	Not present	Brendan - high risk list	15	4	11		0	
59	56	<i>Orgyia antiqua</i>	Not present	Brendan - high risk list	124	0	120		4	

[illegible]

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y							
1	Table S4		Species level dsatabase searches for microbial pathogens																													
2	Number of allowed PCR primer mismatches															Within family specificity										Accuracy			Specificity			
3	#	Common name/ Disease	Scientific name	Type	Source	Status	Barcode type	Sequences*	0	1	2	3	4	No amp found	Mismatch location	Usable barcodes ? ¹	% ident in species	% ident in family	Mega alignment [†]	Seq ID %	Consensus barcode?	BOLD search	nBLAST	final number of barcodes for list	notes for specificity of COI/ primer mismatches							
4	1	Dano foliar pini	<i>Phytophthora pinifolia</i>	Oomycete	Forestry high risk list	Not present	COI	3	0	0	0	0	0	-	3 rev, 2for	0	-	-	-	-	-	-	-	0	Manually checked again on GenBank Gene. Same result							
5	2	Pine pitch canker	<i>Fusarium circinatum</i> <i>Fusarium oxysporum</i> and <i>F. solani</i>	Fungus	Forestry high risk list	Not present	ITS- GF assay	43	7	7	7	7	7	36	NA	7	100%	100%	-	100%	Y	100% <i>Fusarium</i> , genus level including 100% <i>fusarium circinatum</i>	Various <i>fusarium</i> species 100% including <i>circinatum</i>	1	Other <i>fusarium</i> also 100% - to be expected							
6	3		<i>Fusarium oxysporum</i>	Fungus	Closely related/ often isolated	Present	ITS- GF assay	2124	87	91	92	92	98	2026		15	95-100%		15	95-100%	N	100% <i>Fusarium oxysporum</i> , uncultured <i>fusarium</i>	11									
7	4		<i>Fusarium solani</i>	Fungus	Closely related/ often isolated	Present	ITS- GF assay	1803	12	14	15	15	16	1787		97	96-100%		97	96-100%	N	98-100% <i>Fusarium solani</i> , <i>F. oxysporum</i> , <i>F. keratoplasticum</i> etc	15									
8	5	Western gall rust	<i>Endocronartium harknessii</i>	Fungus	Forestry high risk list	Not present	ITS- GF assay	42	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	0								
9	6	Sudden oak death	<i>Phytophthora ramorum</i>	Oomycete	Forestry high risk list	Not present	ITS- GF assay	6	0	0	0	0	5	1	3 rev 3; 1f	0	-	-	-	-	-	-	-	0								
10	7	Brown spot needle blight	<i>Lecanosticta acicola</i>	Fungus	Forestry high risk list	Not present	ITS- GF assay	8	2	2	2	2	2	1	NA	2	100%	-	2	100%	Y	NA	100% <i>Lecanosticta acicola</i> , 99.03% <i>Lecanosticta sp</i>	1	Other <i>lecanosticta</i> 99% and below							
11	8	Pine wilt nematode	<i>Bursaphelenchus xylophilus</i>	Nematode	Forestry high risk list	Not present	COI	132	0	0	0	99	99	33	1f/2rev	99	95.97-99.19	-	12	95.97-99.19	N	NA	12									
12	9		<i>Heterobasidion annosum</i>	Fungus	Forestry high risk list	Not present	ITS- GF assay	25	0	0	8	8	8	17	2 rev	8	99.62-100%	-	8	99.62-100%	N	NA	99.62-100% <i>Heterobasidion annosum</i> ; <98.48 <i>Heterobasidion irregulare</i>	3								
13	10		<i>Colletotrichum acutatum</i>	Fungus	Forestry high risk list	Present	ITS- GF assay	474	0	11	11	11	11	463	1 rev	11	95.74-100%	75.3-100%	11	95.74-100%	N	NA	Collectotrichum species 100% (<i>florinae</i> , <i>acutatum</i> , <i>lupini</i>)	3								
14	11	Lupin blight	<i>Colletotrichum gloeosporioides</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	418	0	19	20	21	21	397	rev 1	21	74.7-100%		21	74.7-100%	N	NA	Colletotrichum gloeosporioides 100%, <i>C. plurivorum</i> , <i>C. philoxeroidis</i> 99.21%	15								
15	12	Red needle cast	<i>Phytophthora pluvialis</i>	Oomycete	Forestry high risk list; EPPQ	Present	COI	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
16	13	Red band needle blight	<i>Dothistroma septosporum</i>	Fungus	Forestry high risk list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
17	14		<i>Corinectria fuckeliana</i>	Fungus	Forestry high risk list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
18	15	Bacterial leaf scorch	<i>Xylella fastidiosa</i>	Bacteria	MPI	Not present	16S	2	2	2	2	2	2	0	-	2	100%	100%	1	100%	Y	NA	100% <i>Xylella fastidiosa</i> , 100% <i>Xylella takwanensis</i>	1	Nothing in BOLD. 2 sequences from stain ICMP 15197 in GenBank gene repository for NZ_CP118539.1							
19	16	Dutch elm disease	<i>Ophiostoma novo-ulmi</i>	Fungus	Notifiable organisms order 2016	Not present	ITS- GF assay	31	0	0	0	0	0	31	-	0	-	-	-	-	-	-	-	0								
20	17	Eucalyptus rust	<i>Austropuccinia psidii</i> (synonym <i>Puccinia</i>)	Fungus	Notifiable organisms order 2016; EPPQ	Present	ITS- GF assay	39	0	0	0	3	20	19	3 rev, 1f	20	99.26-100%	-	20	99.26-100%	N	NA	100% <i>Austropuccinia psidii</i>	4								
21	18	Brown rot	<i>Phytophthora lateralis</i>	Oomycete	Notifiable organisms order 2016	Not present	ITS- GF assay	36	0	0	0	0	0	-	3 rev	0	-	-	-	-	-	-	-	0								
22	19	Black stain root disease	<i>Ophiostoma wageneri</i> (<i>Grosmannia wageneri</i>)	Fungus	Notifiable organisms order 2016	Not present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
23	20	Scleroderis canker	<i>Gremmeniella abietina</i>	Fungus	Notifiable organisms order 2016	Not present	ITS- GF assay	51	44	44	44	44	44	6	-	44	94.93-100%	<95%	44	94.93-100%	N	NA	100% <i>Gremmeniella abietina</i> , <95.41% <i>Gremmeniella balsamea</i>	6								
24	21	Acacia rust	<i>Uromycladium spp. (7 species)</i>	Fungus	NZFFA forest disease list	Present			0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
25	22	Native heart-rot fungus of hardwood	<i>Agrocybe parasitica</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	2	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
26	23		<i>Alantophomopsisella pseudotsugae</i> (<i>Alantophomopsis pseudotsugae</i>)	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
27	24	Plane anthracnose	<i>Apiognomonia platani</i> (<i>Apiognomonia veneta</i>)	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
28	25	Barron Road Syndrome	<i>Fungal complex</i>	Fungus	NZFFA forest disease list		ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
29	26	Black stain root disease	<i>Leptographium spp.</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								
30	27		<i>Botryosphaeria dothidea</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	705	12	12	12	12	12	693	-	12	98.83-100%	<99.61%	12	98.83-100%	N	100% <i>Botryosphaeria dothidea</i>	99.61-100% <i>Botryosphaeria dothidea</i> , <99.61% <i>B. dothidea</i> , <i>B. sinensis</i>	3								
31	28		<i>Botrytis cinerea</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	270	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0								

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
32	29	Cladosporium leaf spots	<i>Cladosporium</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	Only sequences for beta-tubulin on GenBank
33	30	needle-cast	<i>Cyclaneusma minus</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
34	31	Cypress canker	<i>Seiridium unicomae</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
35	32	Cypress canker	<i>Seiridium cardinale</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
36	33	Cytospora disease	<i>Cytospora sp</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	100% Teratosphaeria eucalypti, 99.55% T. destructans, 98.19% T. viscida, 98.56% T. pseudoeucalypti
37	34	Diplodia leader dieback etc	<i>Sphaeropsis sapinea (Syn: Diplodia pinea)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
38	35	Elm leaf spot	<i>Phloeospora ulmi</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
39	36		<i>Gloeopeniophorella sacra (Dextrinocystidium sacratum)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
40	37	Fungus disease of Eucalyptus seed	<i>Hainesia lythri (Pilidium lythri)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	Nothin at species level for 3 main hits associated with pine
41	38	Disease of Ash in New Zealand	<i>Hysterographium fraxini</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	1	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
42	39	Jungliahnia root disease	<i>Jungliahnia vineta (Lividopora vineta)</i>	Fungus	NZFFA forest disease list	?	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
43	40	Kabatina thujae	<i>Kabatina thujae</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
44	41	septoria leaf blight	<i>Kirramyces eucalypti (Teratosphaeria eucalypti)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	3	1	1	1	1	1	2	-	1	NA	NA	1	100%	Y	NA	100% Teratosphaeria eucalypti, 99.55% T. destructans, 98.19% T. viscida, 98.56% T. pseudoeucalypti	1	Nothin at species level for 3 main hits associated with pine
45	42		<i>Lepteutypa podocarpi</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
46	43		<i>Leptographium spp.</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay		0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
47	44		<i>Lophodermium spp.</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay		0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
48	45		<i>Lophodermium conigenum</i>			Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	100% Phaeocremioniu rubrigenum, P. scolyti, 97.01% P. luteum
49	46		<i>Lophodermium pinastri</i>			Present	ITS- GF assay	-	0	0	0	0	0	-	-	1	-	-	-	-	-	-	-	1	
50	47		<i>Lophodermium hiltzer</i>			Present	ITS- GF assay	-	0	0	0	0	0	-	-	2	-	-	-	-	-	-	-	2	
51	48		<i>Meria laricis (Rhodocline laricis)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
52	49	Oak Powdery Mildew	<i>Microsphaera alphitoides (Erysiphe alphitoides)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	67	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	99.31-100% Phaeolus schweinitzii, <99.6% P. hispidus, P. himalayanus
53	50	nectria flute canker	<i>Neonectria fockeliana (Corinectria fockeliana)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
54	51		<i>Pachysacca pusilla</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
55	52	Phaeocremioniu rubrigenum	<i>Phaeocremioniu rubrigenum</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	18	4	4	4	4	4	14	-	4	91.35-100%	NA	4	91.35-100%	N	NA	100% Phaeocremioniu rubrigenum, P. scolyti, 97.01% P. luteum	2	
56	53	Swiss needle cast disease of Doug	<i>Phaeocryptopus gaeumannii (Nothophaeocryptopus gaeumannii)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	For the pathovar there are only discontinued gene sequences on GenBank (withdrawn by genbank staff), no 16S broadly either
57	54	exotic wood decay fungus	<i>Phaeolus schweinitzii</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	9	1	1	1	1	1	8	-	1	NA	NA	1	100%	Y	NA	99.31-100% Phaeolus schweinitzii, <99.6% P. hispidus, P. himalayanus	1	
58	55	Elm leaf spot	<i>Phloeospora ulmi</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
59	56		<i>Phomopsis juniperivora (Diaporthe juniperivora)</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
60	57		<i>Phytophthora agathadictya</i>	Oomycete	NZFFA forest disease list	Present	COI	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	Same two barcodes that worked at the genus level
61	58	Kauri dieback	<i>Phytophthora kernoviae</i>	Oomycete	NZFFA forest disease list	Present	COI	11	0	0	0	0	0	-	3 rev	0	-	-	-	-	-	-	-	0	
62	59	totara needle blight	<i>Phytophthora podocarpi</i>	Oomycete	NZFFA forest disease list	Present	COI	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
63	60	Poplar anthracnose	<i>Marssonina species</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay		0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
64	61		<i>Marssonina brunnea</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	For the pathovar there are only discontinued gene sequences on GenBank (withdrawn by genbank staff), no 16S broadly either
65	62		<i>Marssonina castanei</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	-	0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	
66	63	Bacterial leaf spot and stem canker	<i>Pseudomonas syringae pv. syringae</i>	Bacteria	NZFFA forest disease list	Present	COI	8	0	0	0	0	0	-	3 rev	0	-	-	-	-	-	-	-	0	
67	64	Rhizosphaera spp.	<i>Rhizosphaera spp.</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay		0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	Same two barcodes that worked at the genus level
68	65		<i>Rhizosphaera kalkhoffii</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	18	2	2	2	2	2	16	-	2	97.2-100%	NA	2	97.2-100%	N		97.6-100% Rhizosphaera sp; 97% Phaeocryptopus	2	
69	66	Rugonectria canker fungus	<i>Rugonectria sp</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay		0	0	0	0	0	-	-	0	-	-	-	-	-	-	-	0	

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	Table S5 Genus level dsatabase searches for microbial pathogens																							
2	#	Genus	Type	Source	Status	Barcode type	Sequence s*	0	1	2	3	4	No amp found	Mismatch location	Usable barcodes ? ¹	% ident in species	% ident in genus	Mega alignment	Seq ID %	Consensus barcode?	BOLD search	nBLAST	final number of barcodes for list	notes for specificity of COI/ primer mismatches
3	1	Allantophomopsiella	Fungus	NZFFA forest disease list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	0	A monotypic genus, so no species hits = no genus hits
4	2	Apiognomonina	Fungus	NZFFA forest disease list	Present	ITS- GF assay	57	0	0	0	0	0	57	-	0	-	-	-	-	-	-	-	0	
5	3	Leptographium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	380	7	7	7	7	7	373	NA	7	NA	77.8-100%	7	77.8-100%	N	93.33-100% Leptographium, 81-92% Grossmania, 81%-Ophiostoma	4	Several species associated with pine in NZ	
6	4	Phloeospora	Fungus	NZFFA forest disease list	Present	ITS- GF assay	1	0	0	0	0	0	1	-	0	-	-	-	-	-	-	-	0	
7	5	Seiridium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	0	
8	6	Agrocybe	Fungus	NZFFA forest disease list	Present	ITS- GF assay	174	4	4	4	4	4	170	NA	4	NA	64-100%	4	64-100%	N	94-100% Agrocybes	4	No top hits for parasitica	
9	7	Austropuccinia (synonym Puccinia)	Fungus	Notifiable organisms order 2016; EPPO	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	0	A monotypic genus, so no species hits = no genus hits	
10	8	Botryosphaeria	Fungus	NZFFA forest disease list	Present	ITS- GF assay	880	14	14	14	14	14	866	NA	14	NA	79-100%	14	79-100%	N	Botryosphaeria 98.46-100%	5		
11	9	Botrytis	Fungus	NZFFA forest disease list	Present	ITS- GF assay	518	0	0	0	0	0	518	-	0	-	-	-	-	-	-	-	0	
12	10	Bursaphelenchus	Nematode	Forestry high risk list	Not presen	COI	521	0	0	0	0	0	521	-	0	-	-	-	-	-	-	-	0	
13	11	Ceratocystis (Sphaeronaemella)	Fungus	EPPO	Present	ITS- GF assay	655	35	36	36	36	36	619	-	34								19	
14	12	Cladosporium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	800	11	226	227	230	231	569	rev 1	232	4	66-100%	232	66-100%	N	Cladosporium , ascomycota, davidiella 100%	31	Multiple cladosporium speices in New Zealand	
15	13	Colletotrichum	Fungus	Forestry high risk list	Present	ITS- GF assay	2495	0	91	99	102	102	2393	rev1, f1	102	NA	85-100%	102	85-100%	N	99.61-100% Colletotricum	42		
16	14	Corinectria	Fungus	Forestry high risk list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	0	New genus from 2017 closely related to Neonectria	
17	15	Neonectria (Corinectria)	Fungus	NZFFA forest disease list	Present	ITS- GF assay	229	13	15	17	17	20	209	1/2 f	17	NA	76-100%	17	76-100%	N	99.6-100% Neonectrina spp, <99.06% uncultured fungus	11	Same species as above but using a different genus search (neither species level had any luck)	
18	16	Cyclaneusma	Fungus	NZFFA forest disease list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	0		
19	17	Cytospora	Fungus	NZFFA forest disease list	Present	ITS- GF assay	633	4	4	4	4	4	629	NA	4	NA	86-100%	4	86-100%	N	85-100% cytos	4		
20	18	Diplodia (Sphaeropsis)	Fungus	NZFFA forest disease list	Present	ITS- GF assay	883	4	4	7	7	7	876	NA	4	NA	82-100%	4	82-100%	N	99.21-100% Di	3		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	
		<i>Dothistroma</i>																				98.61-100% Dothistroma, mostly Pini. <97.3% Aamycosphae rella, 98.62% Mycosphaerel a			
21	19		Fungus	Forestry high risk list	Present	ITS- GF assay	3		1	1	1	1	2	NA	1	NA	NA	1	NA	NA					
22	20	<i>Endocronartium</i>	Fungus	Forestry high risk list	Not presen	ITS- GF assay	42	0	0	0	0	0	42	-	0	-	-	-	-	-	-	-	0		
23	21	<i>Erysiphe</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	877	25	32	33	33	34	843	1/2 on f	34	NA	72-100%	34	72-100%	N		100% Erysiphe	19		
		<i>Fusarium</i>																						Fusarium oxysporum and F. soilani are primary tree pathogens often identified. Many species in NZ https://www.sciencedirect.com/science/article/pii/S187861461000073#sec3 abundant members of soil diversity	
24	22		Fungus	Forestry high risk list	Not presen	ITS- GF assay	11,833		212	223	226	226	241	11592	f1	239	NA	66-100%	239	66-100%	N		100% Fusarium sp	66	
25	23	<i>Gloeopeniophorella</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	5	0	0	0	0	0	5	-	0	-	-	-	-	-	-	-	0		
26	24	<i>Gremmeniella</i>	Fungus	Notifiable organisms order 2(	Not presen	ITS- GF assay	66		55	55	55	55	11	n	55	NA	96-100%	55	96-100%	N		93.5-100% Gre	8		
27	25	<i>Heterobasidion</i>	Fungus	Forestry high risk list	Not present	ITS- GF assay	148		0	0	36	36	36	112	rev 2	36	NA	96-100%	36	96-100%	N		93.5-100% Het	10	
28	26	<i>Hysterographium</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	5		1	1	1	1	4	-	1	NA	NA	1	100%	NA		100% Hysterog	1	Positive hits for highly abundant soil microbes	
29	27	<i>Kabatina</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	4	0	0	0	0	0	4	-	0	-	-	-	-	-	-	-	0		
30	28	<i>Lecanosticta</i>	Fungus	Forestry high risk list	Not presen	ITS- GF assay	8		2	2	2	2	6	-	2	NA	100%	2	100%	Y		92.14-100% Le	1		
31	29	<i>Lepteutypa</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	0		
32	30	<i>Lividopora</i>	Fungus	NZFFA forest disease list	?	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	0		
																								Broad distribution in NZ, n 21 recorded in nz, 3 species recorded from pine (conigenum, hilitzer and pinastri). No consensus on any barcodes	
33	31	<i>Lophodermium</i>	Fungus	NZFFA forest disease list	Present	ITS- GF assay	309		5	22	22	23	27	282	f0-3	23	NA	66-100%	23	66-100%	N		94.2-100% Lophodermiu m, <92% Leotiomycete s	23	

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
34	32	Marssonina	Fungus	NZFFA forest disease list	Present	ITS- GF assay	24	5	5	5	5	5	19	-	5	NA	80-100%	5	80-100%	N		77-100% Marssonina, 99.6% Diplocarpon coronariae	3	2 species primarily cause this, hits at the species level. Brunnea arrived 1976, castagnei later. Overlap with Diplocarpon coronariae, a closely related pathogen that causes marssonina blotch in apples
35	33	Meria	Fungus	NZFFA forest disease list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	0	Only comes up with insect specimen samples
36	34	Ophiostoma	Fungus	Notifiable organisms order 2016	Not present	ITS- GF assay	992	62	66	66	66	66	926	0 or 1f/1r	66	NA	58-100%	66	58-100%	N		100% Ophiostoma spp	27	
37	35	Pachysacca	Fungus	NZFFA forest disease list	Present	ITS- GF assay	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	0	
38	36	Phaeoacremonium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	72	14	14	14	14	14	58	-	14	NA	60-100%	14	60-100%	N		93-100% Phaeoacremonium spp	10	
39	37	Phaeocryptopus	Fungus	NZFFA forest disease list	Present	ITS- GF assay	25	3	3	3	3	3	22		3	NA	100%	3	100%	N		aeocryptopus, Phaeocryptopus	3	Nothophaeocryptopus = phaeocryptopus
40	38	Phaeolus	Fungus	NZFFA forest disease list	Present	ITS- GF assay	9	1	1	1	1	1	8	-	1	NA	NA	1	100%	NA		99.3-100% Phaeolus, 98% Polyporus		Top hits are all for Phaeolus schweinitzii
41	39	Phloeospora	Fungus	NZFFA forest disease list	Present	ITS- GF assay	1	0	0	0	0	0	1	-	0	-	-	-	-	-	-	-	0	
42	40	Phomopsis	Fungus	NZFFA forest disease list	Present	ITS- GF assay	307	22	22	23	23	27	280		27	NA	82-100%	27	82-100%	N		98-100% Phomopsis, Diaporthe	19	Removed 5 for too many reverse mismatches. Phomopsis is an anamorph of Diaporthe
43	41	Phytophthora (COI)	Oomycete	NZFFA forest disease list	Present	ITS- GF assay	588	0	0	0	0	0	588		0	-	-	-	-	-	-	-	0	Too many reverse mismatches
44	42	Phytophthora (ITS)	Oomycete	NZFFA forest disease list	Present	COI	1,325	0	0	0	0	66	1259	1f/3, R3	19	NA	93-100%	19	93-100%	N		95-100% Phytophthora	5	Pushing it on the mismatches. 3 on reverse and up to 3 on forward
45	43	Pilidium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	14	1	2	2	2	2	12	1f	1	NA	100%	1	NA	NA		85-100% Pilidium, Discohainesia	1	
46	44	Pseudomonas	Bacteria	NZFFA forest disease list	Present	COI	578	12	13	13	13	13	565	o or 1f	13	NA	91-100%	13	91-100%	N		100% Pseudomonas, Ectop	10	
47	45	Raffaelea	Fungus	EPPO	Not present	ITS- GF assay	30	0	0	0	0	0	30	-	0	-	-	-	-	-	-	-	0	
48	46	Rhizosphaera	Fungus	NZFFA forest disease list	Present	ITS- GF assay	18	2	2	2	2	2	16		2	97.2-100%	NA	2	97.2-100%	N		97.6-100% Rhizosphaera sp; 97% Phaeocryptopus	2	Same two barcodes that worked at the species level

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
49	47	Rugonectria	Fungus	NZFFA forest disease list	Present	ITS- GF assay	4	0	0	0	0	0	4	-	0	-	-	-	-	-	-	-	0	
		Sarcostroma	Fungus	NZFFA forest disease list	Present	ITS- GF assay																84-100% Sarcostroma, 87% Seimatosporium, Pestalotiopsis, Ellurema		
50	48						4	1	1	1	1	1	3	-	1	NA	100%	1	NA	NA			1	
51	49	Seiridium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	16	1	1	1	1	1	15	-	1	NA	100%	1	NA	NA	istononappendiculata, 93	0	No hits for Seirdium	
		Sporothrix	Fungus	NZFFA forest disease list	Present	ITS- GF assay																High similarity to Ophiostoma, Sporothrix are anamorphs of above		Vectored by Platypus borer. 4 species in aus/nz https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2024.1382508/full though this could be the human relevant strains
52	50						345	22	23	23	23	23	322		23	NA	77.82-100%	23	77.82-100%	N			11	
53	51	Stigmia	Fungus	NZFFA forest disease list	Present	ITS- GF assay	5	0	0	0	0	0	5	-	0	-	-	-	-	-	-	0		
		Teratosphaeria	Fungus	NZFFA forest disease list	Present	ITS- GF assay																91-100% Teratosphaeria, 91% Colletogloeopsis		
54	52						439	116	118	118	119	119	320		119	NA	60-100%	71	60-100%	N			71	
55	53	Uromycladium	Fungus	NZFFA forest disease list	Present	ITS- GF assay	28	0	0	0	0	0	28	-	0	-	-	-	-	-	-	0		
56	54	Xylella	Bacteria	MPI	Not presen	16S	0	0	0	0	0	0	0	-	0	-	-	-	-	-	-	0		
57	55	Junghuhnia (Lividopora)	Fungus	NZFFA forest disease list	?	ITS- GF assay	7	0	0	0	0	0	7	-	0	-	-	-	-	-	-	0	Hits when search for Junghuhnia	