

**The Long Term Housing, Maintenance and Scientific Curation of the
National Fruit Collections**

Defra Project code GC0147

Annual Report 2018/19

Appendix 3

Assessment of the New Pear Collection (Pear NFC 3) – update 2019

The current pear collection (NFC 3) was repropagated during 2006-7 and trees were planted early during 2008. With a view to removing the old collection (NFC 2) an assessment was carried out to verify that all of the accessions to be retained were present and correct in the new plot (*note: 'correct' in this instance meant 'matching the representative tree in NFC 2' rather than necessarily indicating 'trueness to type'*). The report was formally submitted to Defra as Appendix 3 to the GC0143 project report in September 2014 and is updated here to address any remaining queries.

As reported previously (NFC Advisory Committee meeting 18th Jan 2012) a small number of accessions were removed from the collection during the repropagation process; these accessions had been proposed by the previous curators to either be falsely labelled and/or duplicates of other accessions in the NFC (which were themselves retained) and this was confirmed in the analysis by East Malling Research (EMR) as part of Defra project GC0139. Further to this, a number of outstanding curational queries and additional suspected duplicates, raised through analysis of the old collection by EMR (Defra project GC0140), have been addressed below.

A series of accessions (generally, known sports and clones of varietal types) were expected to remain indistinguishable within the collections by DNA analysis and these were therefore checked morphologically alongside the items raised in the original fingerprinting. Despite some complication caused by differences attributed to tree age, health, and potentially soil (fruit in NFC 3 were in general larger, later ripening and more highly coloured than those in NFC 2) it was judged that most of these sports and clones had been reliably propagated given that the DNA analysis was sufficient to type them to their base variety. Specific queries from this process are addressed below.

DNA analysis was then carried out to check the status of the new collection. As previously discussed, (NFC Advisory Committee meeting 11th July 2012) some difficulties were found in the initial approach to carry out this analysis in a purely objective manner. Principally, the attempts to compare only scored data were hampered by the inconsistency of absolute SSR scores and allele calling across different analyses, the need to adjust data to fit previously available datasets (which had been called, scored and rounded) and instances of 'allelic dropout' either creating missing data, or on occasion additional data, which were difficult to judge against the scored results.

Consequently, an approach was taken to allow a more subjective input to be brought to the analysis. DNA was extracted from both collections (NFCs 2 and 3) and analysed in parallel for comparison. SSR profiles (as described by Evans et al., 2009, *Pyrus. Acta Hort.* [ISHS] 814: 103-106 and as per GC0139/40) were produced for all samples. Each accession was compared across the two collections independently using each of three multiplexes containing four markers per multiplex. This method allowed an initial subjective comparison of profiles to be carried out before complementing with a more objective comparison of scored allele data in relation to the original data (from GC0140) where necessary.

Initial scoring was therefore by subjective comparison. Profiles from corresponding trees were compared and judged as either matching/mismatching/failed, or noted for analysis by allele scoring in the case of apparent complexity or where the reaction for the counterpart tree had either failed or had not been included in the analysis (a few trees were lost before this process was carried out). Accessions in the new collection which were judged to match their counterpart in the old collection

across all multiplexes (allowing for occasional missing data but not for conflicting data) were accepted as present and correct (see example below):

Example 1 – Accessions judged to be matching across all multiplex reactions

Accession 2001-053 matches across all reported markers (*note: one additional allele which was scored in the original dataset is missing in both samples*)



Accessions which were without counterparts in the old collection, or where the multiplex had failed for the old accession, were then compared by scoring and comparing allele sizes to the original data. Prior to this, to account for the expected differences between rounds of analysis, a set of 'standards' which had been run across multiple plates were used to estimate an adjustment or translation factor (*note: the standards used in this case were not as per the ECPGR recommendations [Evans et al., 2009] but were selected to better cover the range of alleles expected within the collection, since the ECPGR set had been selected without the benefit of data scored across the whole NFC collection and consequently covered less of the range of potential allele sizes. In addition, the ECPGR standard trees are sited at Brogdale and the original data had therefore already been standardised against these.*) Given that the allele number and size range included between 13 and 32 alleles per marker and covered between 36 and 89bp per marker (although noting that the frequency distribution across these alleles is far from even) this translation factor was taken as a reasonably reliable way to estimate the equivalent alleles and compare data. Further to this, the range of the translation factor across different alleles for any given marker was generally found to lie within a single integer (which again, given that the original data had been rounded to the nearest whole allele value was not unexpected). Using these translation factors (below) the scored and checked data were compared to the original dataset.

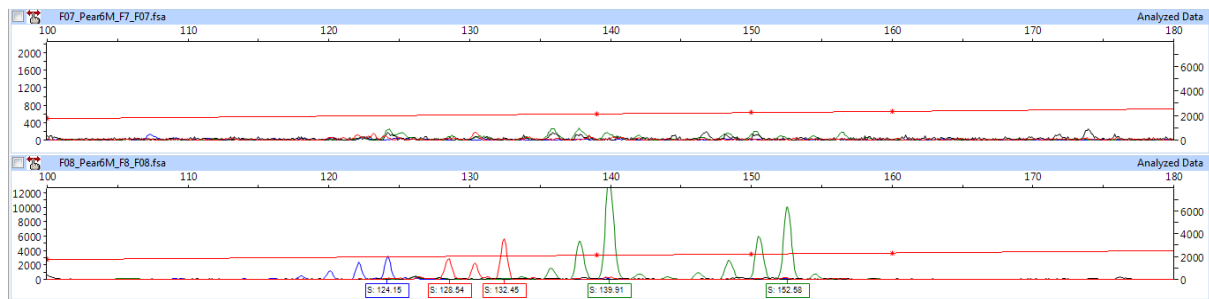
Adjustment factor used for each marker

Multiplex Small		Medium		Large	
Marker	Adjustment	Marker	Adjustment	Marker	Adjustment
EMPC11	-1.5	GD96	-1.5	CH01d08	-2.0
EMPC117	-3.0	CH01d09	-2.0	CH01f07a	-2.5
GD147	-4.5	CH02b10	-2.5	CH04e03	-2.0
CH05c06	-1.5	CH03d12	-1.5	CH03g07	-4.0

Scored accessions were judged as matching/part matching or mismatching in comparison to the original data. Again, some alleles were missing, and a few occasional differences of a single base pair (generally assumed to be due to differences in selection and/or generation of a-addition peaks) were noted after translation. These tended to be associated with individual markers, were generally smaller differences than the allele intervals in the original dataset (i.e. were not large enough to suggest a match the next sized reported allele) and were therefore judged to remain the best match. In the majority of cases and markers where accessions were judged as matching, the translation factor aligned alleles directly to the original dataset. Additionally, in almost all cases, where accessions were judged as matching, their scored data were more than sufficient to identify a single profile from the original dataset (with the obvious exception of the clonal groups and sports).

Example 2 – Accessions identified as correct by scoring

The old collection sample profile (F07) failed for accession 1951-114 but the new collection sample (F08) was confirmed as correct by scoring alleles, translating and comparing to the original data (*note: within this multiplex, one marker failed to report in the new sample*).



In addition to those scored as matching, a series of profiles which were either judged as mismatches or to potentially contain mixed samples (generally identified by an increased number of alleles and deemed to be caused by potential cross-contamination) were scored. Amongst these, a systematic error across plates 8 and 9 was identified for the medium multiplex; plates 8 and 9 had been either swapped or mislabelled. This was initially identified due to an anomaly whereby a small number of 'false' samples had profiles mismatching in only two of three multiplexes on plate 8 whilst the reverse was the case on plate 9. Further scoring across both plates confirmed that they had been swapped and after re-adjustment, the scored profiles systematically aligned across both whole plates (*note: it was not possible to trace this error but it could have been a mix-up or mislabelling during either PCR setup or analysis of the profiles by the service provider*).

Fourteen individual samples were noted to contain possible cross contaminants (*note: during the reaction setup a small number of samples were handled twice, specifically in cases where wells failed to seal fully and the reaction dried up, or where some templates from poor DNA extractions were re-extracted and in-filled into their original position. This double handling created potential for pipetting errors which were judged to be the likely reason for contaminants.*) In nine cases the potential contamination was in the old collection sample which was being used for comparison and in each of these cases the new profile was identified as correct by scoring. In five cases the new collection sample appeared to contain cross-contamination: in two of these the contaminant was identified as matching the profile of a neighbouring well by scoring all alleles and in one case the contaminant was not identified, but contamination was only found in one of the three multiplex reactions; in all three of these cases the samples contained all of the correct alleles for their expected accession in addition to the contaminants and since no alternative match could be found these were accepted as correct. In the two remaining cases it was either not possible to identify the contaminants, or the other multiplexes largely failed, and these were grafted for safety and re-tested. Both of these samples were confirmed as being present and correct after re-extraction and analysis from the same leaf sample.

Example 3 – Identification of contaminants in neighbouring samples

Accession 1949-129 (A11/E11) contained cross contamination from the neighbouring sample (accession 2001-051 (B11/F11)). Correct alleles matching A11 and E11 are labelled in the first image and contaminating alleles, matching the neighbour (B11/F11) are labelled in the second.



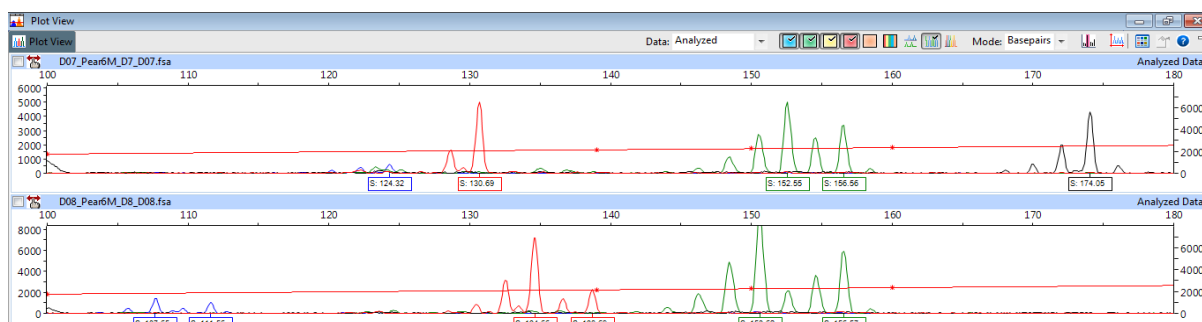


Ten samples were still judged to be false after this re-investigation: six were found to match Doyenne du Comice, presumed to be interstock growth, one was found to be William’s Bon Chretien and one (accession 1948-426) was found to match the neighbouring tree in the new collection (a possible collecting error in the new samples), a further two samples were deemed false but unresolved. One of these (1927-078 Laxton’s Early Market) had already been suspected as a potential mixed plot in the new collection; the second tree was therefore also included in the initial analysis and found to be correct. In the remaining nine cases, trees were grafted for safety and further checks were carried out.

Re-extraction and analysis from the same leaf sample confirmed the two unresolved samples as present and correct. Six new samples were collected from the second tree of the false accessions: four were found to be correct; tree 16_6 Ayresshire Lass was found to be false, but could not be identified within the original profiles and tree 17_14 Summer Bergamot was also found to be a Comice interstock. The original tree from accession 1948-426 was also re-tested and found to be correct (confirming the likely leaf collection error in the first round). For one accession (1958-138) the second tree remained untested.

Example 4 – False samples identified by scoring

Accession 1961-035 in the new collection (D08) was judged as mismatching and identified as matching Doyenne du Comice by scoring (*note: one marker failed to report in the new sample*)



In two further cases the reactions largely failed but presented a few possibly mismatching alleles. Both of these were grafted for safety and samples were re-analysed. Subsequent re-extraction and analysis from the same leaf sample was able to confirm both samples as present and correct.

A number of individual reactions were judged to have failed (approximately 5% of reactions from the new tree samples) and these were generally treated as missing data. For most accessions where one multiplex had failed, the other multiplexes were successful and sufficient data were available to identify the correct profile by scoring; in a single sample two multiplexes failed and the third was only able to narrow down to five possibilities, although one of these was the expected profile and this was accepted as probably correct overall; re-analysis of the same sample produced profiles sufficient to identify the correct profile from the five possibilities (and the remainder of the collection). In three samples the reactions across all multiplexes were poor and since these provided insufficient evidence these were grafted for safety and re-extracted/analysed along with the false and suspect samples. In total sixteen samples were grafted for safety and re-tested. Re-extraction and analysis of the three samples confirmed all three as present and correct

In summary: 395 of 498 accessions were judged to match in all multiplexes and were initially accepted as correct; a further 68 of the remaining 103 were accepted as correct based on two matching multiplex sets and a single failed or part scored multiplex set which together provided sufficient data to uniquely identify the expected profile (including the accession that was found correct for tree 2 only [1927-078 Laxton's Early Market]); 19 of the remaining 35 were accepted where, despite having one or fewer complete matching multiplexes sufficient data were available to uniquely (with one exception) identify the expected profile in the original data (the one exception was later confirmed as correctly matching only one possible profile); 16 were not fully accepted in the initial analysis and these were identified for grafting and re-/further testing.

Of the unresolved samples that were re-tested, all 9 were confirmed as present and correct. Of the remaining 7 that were grafted because Tree 1 was originally identified as false: Tree 2 was identified as correct in 4 cases (including one case where Tree 1 was also found as correct, confirming a likely collecting error in the initial analysis); Tree 2 was found as false but unidentified in one sample (accession 1947-012 Ayreshire Lass) and Tree 2 was found to be Comice in a second (accession 1948-185 Summer Bergamot); one accession was not re-tested at Tree 2. A single accession (1927-078 Laxton's Early Market) was confirmed to have a mixed plot in the new collection with one tree (Tree 2) correct and one (Tree 1) false (as a likely grown out Doyenne du Comice interstock) in the initial analysis.

Unfortunately, for two of the accessions in which grafts had originally reverted to the interstock (1961-035 Moonglow and 1948-185 Summer Bergamot) grafting remained intractable, the accessions being represented by only a single stunted tree with little new growth in the old collection. 1961-035 Moonglow was found to be correct at 13_6 (Tree 2) in the new collection and this can be used as a source of graftwood but 1948-185 Summer Bergamot was found to be false (both trees in the new collection were identified as the Comice interstock, indicating that the original graft had failed and presumably reflecting the state of the original tree). Summer Bergamot will therefore have been lost from the collection.

The following actions were therefore recommended:

Tree 1 for accessions 1975-124 Eyewood, 1976-154 Louise Bonne of Jersey and 1958-138 Bianchettone should be replaced in the new collection with the re-grafted tree; Tree 2 for 1958-138 Bianchettone should be re-checked (note: the latter action is rendered redundant by the finding below that 1958-138 was indistinguishable from 1948-539 Gros Blanquet and the consequent recommendation of deaccession);

Tree 1 for accession 1947-012 Ayreshire Lass should be replaced with the grafted tree and a new tree subsequently grafted to replace Tree 2;

Tree 1 for accessions 1927-078 Laxton's Early Market and 1961-035 Moonglow should be replaced with a new tree grafted from Tree 2;

A healthy replacement tree for the variety Summer Bergamot should be sought from other collections.

With the exception of Louise Bonne of Jersey, for which repropagation is still ongoing, all of the above actions have been carried out. A potential replacement for Summer Bergamot appeared to have survived in the propagation unit and was planted out; this will be checked to confirm whether it is true (as opposed to being another failed graft) in due course.

As above, this analysis presented an opportunity to assess the contents of the Observation Plot as well as addressing a number of specific queries that had arisen from the previous analysis (carried out on the old collection by EMR). In addition it allowed a number of queries which had arisen within the morphological analysis and general observations during curation to be addressed and these are discussed below (with appropriate actions recommended):

Observation Plot

An assessment was made of most of the accessions in the observation plot. The general rationale was to check for mislabelling and duplication with accessions already present in the collection (as had been the major role of the Observation Plot in the past, although this analysis had generally been carried out by morphological assessment). Where accessions were found to be unique they are proposed for accession, where they were not unique they should be removed.

2004-051 Little Swan's Egg and 2002-013 Muirfowl Egg were found to be indistinguishable in the EMR analysis but unique from the remainder of the collection. 2004-051 had already been accessed into the new collection and tree 14_43 was confirmed in this analysis as matching the tree in the observation plot. Two other accessions labelled 'Muirfowl Egg' are also held in the observation plot (2000-108 and 2005-015) the latter distinguished as Muirfowl Egg (Way) in reflection of the donor. These were both included in this analysis and found to themselves be indistinguishable, but different from Little Swan's Egg and also unique from the remainder of the collection – ***2004-051 should remain in the collection as is and one of the other Muirfowl Egg accessions should be brought in from the observation plot (the other should be deaccessed); 2002-013 should be deaccessed.***

1997-013 Jeanne d'Arc in the Observation Plot was found to be unique in the collection both in the EMR and in this analysis. In this analysis two additional alleles were scored (possible failed alleles in

the EMR analysis or potential artefacts). This agrees with the previous inclusion of 1997-013 as a replacement for 1973-329 which was previously found to match Pitmaston Duchess both morphologically and in the EMR analysis and deaccessed – ***trees of 1997-013 can be removed from the Observation Plot.***

2004-052 Round Red Pear was found to be unique in the EMR analysis and accessed into the main collection. In this analysis the new tree was checked and confirmed as unique and correct – ***trees of 2004-052 can be removed from the Observation Plot.***

1996-069 Ogilvy ‘Achan’ in the Observation Plot was identified as matching Autumn Bergamot in the EMR analysis and this was confirmed by morphology – ***1996-069 should be deaccessed from the Observation Plot.***

2005-011 Denny’s Farm, 2007-014 Grey Honey, 2007-016 Goudnap, 2007-013 Maggie Duncan in the Observation Plot were all found to be indistinguishable from 2001-058 Hessele in the EMR analysis and Denny’s Farm was also found to be morphologically similar (slightly later and larger). These findings were confirmed in our initial analysis although 2005-024 Constance Mary was also found indistinguishable where EMR had found it to be different and unique in the collection (possible the sample was cross contaminated in our initial analysis by the neighbouring sample from Denny’s Farm); 2005-024 was checked in later analysis and confirmed to match the EMR profile, and the tree (10_43) in the new collection – ***2005-011, 2007-014, 2007-016, 2007-013 should all be deaccessed and 2005-024 retained in the collection but removed from the observation plot.***

1995-001 Geneting, 2007-003 Beurre Bronze, 2007-015 Craig, 2007-007 Port Allen 2, 2007-010 Port Allen 5 and 2007-017 Rattray were all found to be unique in the collection in this analysis – ***and all should be accessed.***

2006-002 Green Slipper appeared unique in our first analysis although there was some difficulty in scoring and on re-analysis was found indistinguishable from 2001-058 Hessele – ***2006-002 should be deaccessed.***

2007-006 Port Allen 1 was found to be indistinguishable from 2001-093 Green Pear of Yair in both this and the EMR analysis – ***and should be deaccessed.***

2007-008 (5_7)/009 (5_9)/011 (5_13)/012 (5_15) were either not included or failed in our initial analysis. 2007-008 Port Allen 3 was found to be indistinguishable from 2007-007 Port Allen 2 in the EMR analysis though and confirmed as such in the re-analysis; 2007-009 was found to be unique in the re-analysis and 2007-011 and 012 had both been lost prior to this process – ***2007-008 should be deaccessed as 2007-007 is accessed and 2007-009 should be accessed.***

Existing and ongoing verification queries

A number of queries were raised in the initial round of SSR analysis carried out by EMR and further queries had been raised through ongoing curational work at Brogdale. In addition to this, findings were kindly made available (by personal communication) from a report carried out by Joan Morgan and Alison Lean in response to some of the queries raised in the EMR analysis. In cases where

accessions were unexpectedly identified to be indistinguishable, trees were checked morphologically, and where necessary they were checked further in this second round of SSR analysis. Some potential mixed plots were also checked during this process.

1972-090 Belle de Bruxelles and 1935-018 Doyenne Blanc (false) were found to be indistinguishable in the EMR analysis and also in this analysis and are morphologically indistinguishable – **1935-018 should be deaccessed.**

2001-042 Beurre Rance and 1973-289 Unknown S.R. Peart were found to be indistinguishable in the EMR analysis and morphologically identical in this; 1973-289 was originally sent for identification – **can now be identified and should be deaccessed.**

1948-426 Egri and 1948-427 Furedi were found to be indistinguishable in the EMR analysis and were found extremely similar morphologically; Egri appears to have stronger provenance as a variety – **1948-427 should be deaccessed.**

1948-539 Gros Blanquet and 1958-138 Bianchettone were found to be indistinguishable in the EMR analysis and had been previously noted as morphologically similar. Gros Blanquet was found to agree with the description in Hogg; Bianchettone has no known description available – **1958-138 should be deaccessed.**

1972-092 Ferdinand Gaillard and 1948-089 Constant Lesueur were found to be indistinguishable in the EMR analysis and were found almost identical morphologically; both agree with the description for Ferdinand Gaillard. Constant Lesueur has no available description to compare – **1948-089 should be deaccessed.**

2001-061 Jean de Witte and 1935-055 Blickling were found to be indistinguishable in the EMR analysis, confirmed as so in this analysis and appeared identical morphologically. Provenance suggests Jean de Witte is the older cultivar; Blickling may be a previously unknown historical synonym – **1935-055 should be deaccessed and potential synonymy noted.**

1994-003 Liegel's Butterbirne and 1958-132 Virgoloso were found to be indistinguishable in the EMR analysis, were found to be indistinguishable morphologically and verified as matching Liegel's Butterbirne; no published description was available for Virgoloso – **1958-132 should be deaccessed.**

2001-064 Madame Treyve and 2001-088 Howlett 1 were found to be indistinguishable in the EMR analysis and confirmed in this as well as being found indistinguishable morphologically; Howlett 1 is held as an unknown potential seedling – **2001-088 should be deaccessed.**

1975-184 Oldfield and 2002-031 Oldfield were found to be indistinguishable in the EMR analysis and morphologically indistinguishable (fruit were a little smaller in the Perry collection) – **1975-184 should be deaccessed from the main pear collection.**

1977-037 Porporata and 1953-120 Italy 154 were found to be indistinguishable in the EMR analysis and also morphologically; accession details list Italy 154 as a seedling and Porporata as variety with identical parents (presumably this is then the seedling, named) – **1953-120 should be deaccessed and noted in both files.**

1974-244 Rogue Red and 1977-038 Rosired were found to be indistinguishable in the EMR analysis, have been found to be indistinguishable morphologically and verified as both being Rogue Red – **1977-038 should be deaccessed.**

1958-148 Spadona d’Estate, 2001-053 Krystali and 1958-068 Buttira were all found to be indistinguishable in the EMR analysis, were found to be morphologically the same in the collection and are previously thought synonyms; Spadona d’Estate is the longest established name – **2001-053 and 1958-068 should be deaccessed and synonymy noted in files.**

2001-087 Instone 1 was found to be indistinguishable from William’s Bon Chretien in the EMR analysis and confirmed as such in this analysis; was accessed as a seedling tree and retained as unknown in the ICW database – **2001-087 should be deaccessed.**

1948-572 Saint Jean Panachee was found to be indistinguishable from Citron des Carmes and Citron des Carmes Panachee in the EMR analysis and found morphologically to be indistinguishable from the striped form of Citron des Carmes; it was also noted as a synonym in the file – **1948-572 should be deaccessed and synonymy noted.**

1948-423 Buzas Korte and 1948-442 Voros Buza Korte were found to be indistinguishable in the EMR analysis and are supposed sports but fruit were found to be almost identical – **the Voros sport was considered and not found significantly different – 1948-442 should be deaccessed.**

2001-050 Duchesse d’Angouleme, 1949-171 Duchesse Bererd and 1948-062 Duchesse Panachee were all found to be indistinguishable in the EMR analysis and to match in this analysis; 1948-062 is a supposed sport but 1949-171 was recorded as a supposed russeted seedling. Morphologically, 1948-062 was found almost identical to 2001-050 in both collections (i.e. without detectable striping) – **1949-171 should be retained but noted as being identified as a sport, not seedling, 1948-062 was later compared and found to have little striping, but overall more overcolour and should be retained as a potentially valuable sport.**

1952-136 Vermont Beauty and 1974-178 Southworth were found to be indistinguishable in the EMR analysis and were found very similar morphologically apart from Southworth 1974-178 being much earlier ripening; some suggestion that Southworth may have been selected from Vermont Beauty in literature – **1974-178 should be retained as a potential sport as early ripening is of potential value.**

1975-128 Striped Williams was noted to produce fruit with very little striping in the old collection and virtually none in the new collection; both trees were found to match by SSR in this analysis – **it was noted that striping was unstable and attempts to graft from the branch with the most stripy fruit in NFC 2 were unsuccessful - 1975-128 should be deaccessed.**

2001-035 Beurre d’Amanlis and 1948-078 Beurre d’Amanlis Panachee were not noted as indistinguishable in the EMR analysis despite being supposed sports. On checking the original data this was due to a single missing allele (136bp for Ch02b10) which was present but weak in the samples from both old and new trees in this analysis – **accessions should be retained and missing allele noted in original dataset (note: some question remains around the striping on Panachee – if found not stable/present this should possibly be deaccessed in the future).**

1951-127 Beurre Baget was noted through morphological verification to not match its published descriptions but is unique in the collection by SSR analysis – ***1951-127 should be retained but under its accession number.***

2001-092 English Bergamot was not found to match Autumn Bergamot in the EMR analysis despite supposedly being a synonym. This analysis agreed and both found 2001-092 to be unique in the collections – ***2001-092 should be retained but under its accession number and noted as accessed as English Bergamot but found not to be identical to Autumn Bergamot.***

1950-308 Nectarine was not found to match published descriptions morphologically but is unique in the collection by SSR analysis – ***1950-308 should be retained under its accession number and details added in notes.***

1948-749 Reine des Poires was not found to match the published descriptions in Hogg or Downing but does match the accession description in the archive and was unique in the collection by SSR – ***1948-749 should be retained but under its accession number.***

1948-568 Royal d’Hiver was noted in the archive files as being false and the season was found to be wrong (too early) during verification. Spinacarpus has been listed as a published synonym but this accession is different in the collection by SSR analysis and both are unique within the collection – ***1948-568 should be retained but under its accession number.***

2001-077 Suffolk Thorn was found not to match its published descriptions morphologically but was unique within the collections in the EMR analysis – ***2001-077 should be retained but under its accession number.***

2001-079 Swan’s Egg was found not to match its published descriptions morphologically and had been noted as false in the files but was unique within the collections in the EMR analysis – ***2001-079 should be retained but under its accession number (note: this was already listed as ‘unknown’ in the ICW database).***

2001-084 Winter Orange was not found to match the published description in Hogg but was found to be unique in the collections in the EMR analysis – ***2001-084 should be retained but under its accession number.***

2001-031 Bergamotte Heimbourg, 1973-330 Fondante d’Automne & 1975-352 Seigneur Esperen were all found to be indistinguishable in the EMR analysis and were all extremely similar morphologically in this; Seigneur Esperen is noted as a synonym of Fondante d’Automne in Hogg, Bergamotte Heimbourg and Fondante d’Automne have different provenance and further analysis found the accessions to be most likely Fondante d’Automne – ***1975-352 and 2001-031 should be deaccessed.***

2000-140 Dana’s Hovey and 1968-093 Ritson were found to be indistinguishable in the EMR analysis and confirmed in this despite having different provenance. Fruit were also found extremely similar morphologically. Dana’s Hovey has published provenance to being raised by a Mr Dana/Danas and introduced in 1854 and accessions broadly agree with the description; Ritson has less published provenance (to 1914) and is supposedly a seedling. Further morphological checks found the accession to be most likely to be Dana’s Hovey – ***1968-093 should be deaccessed.***

1975-328 Onward and 1951-115 Belle de Soignies were found to be indistinguishable in the EMR analysis but found distinctively different by morphological analysis. In this analysis both trees from the old collection were included as well as trees from the new collection. The new collection tree (16_39) for Belle de Soignies matched both of the the old collection trees (21_27/28) and allele scores matched the profiles for both Belle de Soignies and Onward in the original data; the new collection tree (17_27) for Onward matched both of the old collection trees (21_15/16) but allele scores matched the accession 1975-347 Italy 1437 in both the original dataset and when compared to the scores generated for the old and new trees of Italy 1437 in this analysis. It is then concluded that the profile for Onward in the original dataset is an error and should be as per Italy 1437. Further morphological comparison confirmed 1975-328 and 1975-347 to match and be most similar to the published descriptions of Onward – ***1951-115 should be retained as is and 1975-347 should be deaccessed; SSR data should be corrected for 1975-328.***

1976-177 Alexandrina Bivort and 2001-021 Andre Desportes were found to be indistinguishable in the EMR analysis. The old collection trees for 1976-177 were lost before this analysis but the tree in the new collection was scored as matching the original profile in this analysis; the new tree for 2001-021 matched both its corresponding tree in the old collection and the shared profile in the original data. Further analysis found the accessions to be most similar to descriptions of Andre Desportes – ***1976-177 should be deaccessed.***

1973-322 Merton Pride and 1953-038 Merton Royal were found to be indistinguishable in the EMR analysis, have been found morphologically identical and were confirmed to match in this analysis. Further analysis found the accessions to be most similar to descriptions of Merton Royal – ***1973-322 should be deaccessed.***

2001-032 Beurre Baltet and 1938-010 Laxton's Victor were noted to have potentially mixed and similar plots in the new collection (tree 9_17 being correct and 9_18 appearing different; tree 8_29 appearing correct and 8_30 appearing different; trees 9_18 and 8_30 were noted to appear very similar). Trees 9_17 and 8_29 were included in this analysis and found correct; 9_18 and 8_30 were also included and both found to be Doyenne du Comice – ***a replacement for 9_18 has been grafted from 9_17 and this should be replanted; unfortunately 8_29 was erroneously removed (possibly as the weaker of the pair) and both trees were subsequently replaced using trees standing in the nursery (both of which have since died). Laxton's Victor has consequently been lost and a healthy replacement should be sought if possible.***

1948-098 Doyenne de Montjean was thought to have a possible mixed plot in the old collection resulting in potential for propagation error and both old trees were included in this analysis. 19_35 in the new collection was found to match 24_29 in the old and score as Doyenne de Montjean when compared to the original data. 24_30 from the old collection was also checked and scored as Doyenne de Montjean – ***no action is required.***

1927-078 Laxton's Early Market was noted to appear as a potential mixed plot in the new collection. Trees 8_11 and 8_12 were tested. 8_11 was found to be false (Doyenne du Comice) but 8_12 was found to be true – ***8_11 should be replaced (as above) to correct the mixed plot.***

2001-063 Madame Millet had been proposed as potential mixed plot in the old collection. Tree 2 (8_38) only was included in the EMR analysis and in this analysis both the tree in the new collection

and a sample from Tree 1 (8_37) in the old collection were included and found to match each other as well as the unique profile in the original data (from Tree 2 in the EMR analysis). 1972-008 Madame Ballet had also been proposed as a potential mixed plot (as an alternative, being the neighbouring tree in the old collection) but both trees from the old collection and the tree from the new collection were included and found to match in this analysis – ***no action required.***

2001-040 Beurre Fouqueray was proposed as a potential mixed plot in the new collection and both trees were included in this analysis; 14_21 was found to match the corresponding tree in the old collection and 14_22 was scored as matching the original data – ***no action required.***

1985-003 Hosui had been propagated as an ECPGR reference tree into the new collection (positions 14_45/46) but was thought to be a mixed plot. 14_46 was included in the initial analysis and scored as Doyenne du Comice; 14_45 was included in the re-analysis and scored correctly as Hosui (as per both the EMR profile and a re-analysis from the original tree). However 1_49, which was supposed to be the Hosui accession in the new collection was also found to be false, appearing most similar to the Asian pears although all but Nijisseiki could be excluded by scored alleles (and a number of missing alleles in both profiles could potentially exclude Nijisseiki) – ***tree 14_46 was recommended to be replaced from either 14_45 or 3_8 in the Asian pear trial and 1_49 was also to be replaced (1_50 to be checked further). Unfortunately, 14_45 was erroneously removed and all trees have been replaced from 1_50 (which will need to be confirmed to be correct).***

1973-324 Kieffer 1 and 1975-327 Kieffer 4 were not found to be indistinguishable in the EMR analysis despite being thought to be clones of the variety Kieffer. In the original data, tree 28_41 Kieffer 1 is quite different from Kieffer 4 but indistinguishable from 31_01 Madame Bonneford across the medium and large multiplex scores (which together are more than sufficient to isolate the profiles of Kieffer 1 and Madame Bonneford from the rest of the collection). The alleles reported in the small multiplex for Kieffer 1 do not match the profile for Madame Bonneford but are insufficient to narrow considerably within the dataset. In this analysis Kieffer 1 and 4 match completely, both match their corresponding trees (28_41 and 29_41) in the old collection and also match the profile for Kieffer 4 in the original data apart from a single marker which appeared to report four alleles; Madame Bonneford 21_13 in the new collection matches its corresponding tree 31_01 in the old collection completely and also matches the original profile for Kieffer 1 in all alleles apart from GD96 where it failed to report (i.e. it scores more similarly to the original profile for Kieffer 1 than to the original profile for Madame Bonneford itself). The allele scores for Madame Bonneford in the original small multiplex data (from the EMR analysis) are sufficient to identify only one other accession (02_21 Eva Baltet) with a further single accession matching in all but a missing allele. Kieffer 1 and 4 looked similar, although not identical, morphologically in this analysis. It is therefore assumed that there is most likely to be an error in the original profile; the profile presented from the EMR analysis for Madame Bonneford could potentially be explained by a concatenation of allele scores from Eva Baltet in the small multiplex and Madame Bonneford for the medium and large multiplex; the profile for Kieffer 1 appears complete but is that of the accession Madame Bonneford; Kieffer 1 and 2 should properly match (as per the new analysis) and score as per Kieffer 4 in the original data and Madame Bonneford should not contain the additional small alleles aligned with Eva Baltet – ***accessions should be left as they are and SSR data should be corrected.***

1951-116 Bergamotte de Strycker has some disagreement over its trueness to type but morphological verification in this analysis suggested it was probably true and it is unique in the collection by SSR – ***no action required.***

1935-052 Beurre Alexandre Lucas and 1960-015 Lucas Bronzee were not noted as indistinguishable in the EMR analysis but the original SSR profiles were identical when checked (in agreement with Lucas Bronzee being a sport) – ***no action required.***

20_15 Soldat Laboureur and (19_15) Csatar 1948-424 were found to be indistinguishable in the EMR analysis and also indistinguishable morphologically. Csatar is a published synonym of Soldat Laboureur; only one of these was propagated into the new collection (tree 7_23) and this was found to match both 19 and 20_15 in the old collection in this analysis – ***no action required (note: Soldat Laboureur in the catalogue has an incorrect accession number 1948-425 which is also used correctly for Cserlevelo Csaszar – needs to be corrected to 1975-353 in files).***

1948-751 Herzogin Elsa had been identified as a potential mixed plot in the old collection and both trees were included in this analysis along with the new collection tree. All were found to match and also scored as matching the profile in the original dataset – ***no action required.***

1951-131 Beurre de Beugny has been previously identified morphologically as Bonne de Beugny. The catalogue lists as Beurre but ICW database lists the variety as Bonne; Bonne de Beugny has a published reference – ***1951-131 should be named Bonne de Beugny.***

1958-008 Coscia Tativa was noted as an incorrect spelling of Coscia Tardiva – ***accession name should be altered in records and on label.***

1948-069 Dorsel has no references as a name and despite some question was felt to agree with the description of Dorset in Vercier and Hedrick (archive notes suggest it was thought to be Dorset previously) – ***accession name should be changed to Dorset but verification query acknowledged if possible.***

1972-012 Doyenne de Poitiers had a query over its relationship with a variety of the same name now grown in France. No published descriptions are available to check against – ***query should be noted but accession retained as is.***

1935-022 English Caillot Rosat was found morphologically not to agree with Hogg's description, however Bunyard reports very variable quality; it was felt that this variable quality was reflected in this morphological analysis – ***1935-022 should be retained as is and noted that verification potentially agrees with Bunyard but not with Hogg.***

1968-088 Giant Seckel had been noted through morphological verification to be small/medium in size but is unique in the collection by SSR – ***1968-088 should be retained but possible false verification status noted.***

1968-082 Max Red Bartlett was found morphologically to be bigger, darker and duller red in the new collection. This was in line with a general tendency for the new trees to be larger, less ripe and less brightly coloured. This SSR analysis matched the new and old trees – ***no action required.***

1968-084 Parburton appeared morphologically to have darker, duller green fruit in the new collection but this analysis matches the SSR profile to the old tree – ***no action is required.***

1935-068 Vicar of Winkfield was noted as a synonym of ‘Le Cure’ which is the original name for this variety; the variety is mostly known as Vicar of Winkfield in the UK – ***accession should be retained as Vicar of Winkfield with Le Cure as a listed synonym.***

1994-007 and 1992-012 Graparon (both) was present as two accessions in the old collection but only repropagated into the new collection from 1994-007; previous analysis had been carried out on 1992-012. The profile for the new tree in this analysis largely failed and the sample for the corresponding old tree (1994-007) was, unfortunately, cross contaminated with the neighbouring well (as identified by allele scoring). The profile for 1992-012 was found to match the original data in this analysis (which was carried out on the same tree) and further analysis of the new 1994-007 sample confirmed it as matching the profile of the old 1992-012 accession. This confirms that the two accessions were duplicates and that the 1994-007 accession is present and correct in the new collection – ***no action is required.***

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