First Report of Shot Hole disease on Cherry Laurel (*Prunus laurocerasus*) Caused by *Micrococcus aloeverae* in Ireland

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Cherry laurel (*Prunus laurocerasus* L.) is an evergreen shrub of the Rosaceae family (Kolayli et al. 2003). It is native to the Black Sea coast and South-west Asia where it grows in forest understoreys or as a subdominant tree (Hättenschwiler and Körner 2003). In Ireland it is used for hedging and cultivated in plantations for evergreen foliage and stems which are harvested annually (Whelton 2013).

Shot hole disease of cherry laurel is characterised by brown lesions, red margins and round abscissions, which reduce the quality of leaves and yield of marketable stems. Since the 1980's the disease has been reported regionally within Europe and North America (Roberts 2011). Current opinion is that shot-hole is commonly caused by the bacterial pathogen *Pseudomonas syringae* pv. *syringae* (*Pss*). However, X*anthomonas arboricola* pv. *pruni* also infects cherry laurel (Roberts 2011; Tjou-Tam-Sin et al. 2012).

During an epidemiological study of *Pss*, shot hole symptoms were found on cherry laurel hedgerows cv. Rotundifolia at Belfield in Dublin, Ireland (53°18'33.7"N 6°12'58.6"W), on September 28th, 2017. Sampled leaves (5) were surface sterilized (3.5% NaOCl, followed by 70% ethanol and rinsed 3 times with sterile water) and incubated on water agar at 25°C for 7 days to allow lesion development. Tissue developing lesions were ground with a mortar and pestle and the solution plated on KB agar (King et al. 1948) for 2 days at 25°C. Isolated colonies were circular, pale yellow/cream, shiny, smooth and raised. Examination with a light microscopy revealed spherical, tetrad forming bacteria.

Koch's postulates were fulfilled by inoculation of two plants of cv. Novita, 6 leaves per plant, with $5x10^6$ cfu/ml of bacterial suspension using infiltration with a blunt 1ml syringe (300 µl). Water was infiltrated into leaves as a control. Plants were placed in a growth chamber at 20°C and 85% relative humidity (16:8 h light dark cycle). At 7 dpi necrotic areas were observed surrounded by a halo of water soaking. By 14 dpi an abscission layer formed around water soaked areas on inoculated leaves, which had abscised by 21 dpi. Symptoms were similar to lesions and abscissions observed on the hedgerow with the initial shot hole symptoms. Koch's postulates were also completed on 3 surface sterilized detached leaves cv. Rotundifolia with $5x10^6$ cfu/ml of a bacterial suspension with water as a control. Leaves were placed on water agar inside sealed plastic bags to maintain humidity at 20°C. An abscission layer formed by 21 dpi and by 28 dpi water soaked areas had abscised. This was repeated three times independently. Bacteria re-isolated from infected leaves had the same spherical tetrad forming morphology.

DNA was extracted from the initial and re-isolated bacterial colonies. The conserved region of 16S rRNA genes were amplified using the primers, BAC338F and BAC805R (Yu et al. 2005). Sequences were submitted to GenBank under Accession Nos. MN630641 and MN630642. Blast analysis of these 16S rRNA fragments (410 bp and 399bp) shows 99% identity with *Micrococcus aloeverae* (NR_134088.1). The genome of the re-isolated colony was sequenced using an Illumina platform and submitted to GenBank (PRJNA603651). Phylogenetic analysis revealed 98% identity with *M. aloeverae* (PRJNA526783). *Micrococcus* species have been reported as endophytes (Prakash et al. 2014) and pathogens of horse chestnut (Iakovleva et al.2013) and mango (Rakhashiya et al. 2015). Further work will establish how widespread *M. aloeverae* is as a pathogen of cherry laurel.

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The authors declare no conflict of interest.

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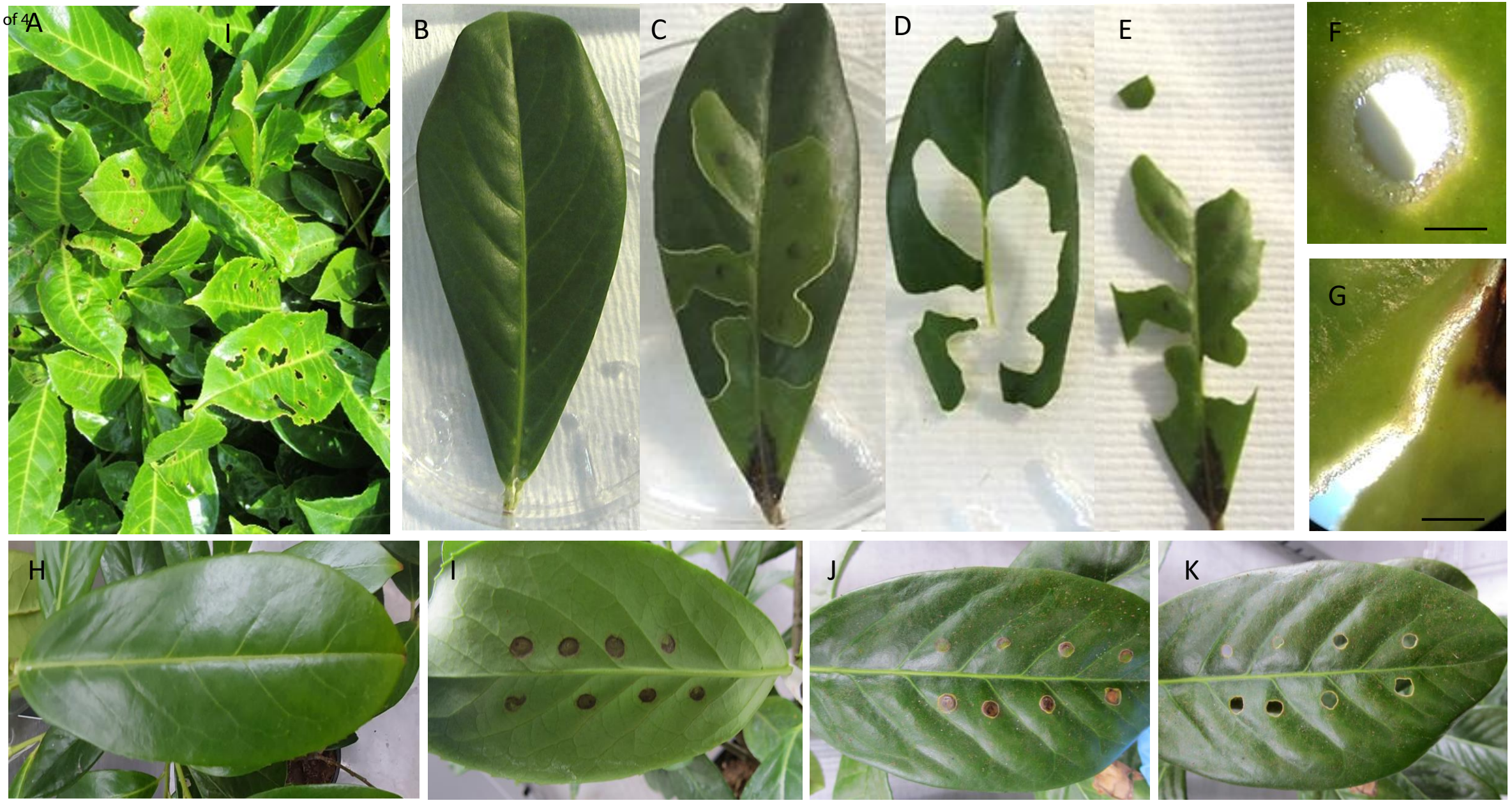

Figure S1. *Prunus laurocerasus* (cherry laurel) (A) hedgerow cv. Rotundifolia displaying shot hole symptoms (B) cv. Novita uninfected detached leaf (C) infected with *Micrococcus aloeverae* detached leaf 21 days post infection (dpi) (D) (E) 28 dpi abscission area (F) (G) abscissions, scale bar =2.5mm (H) uninfected cv. Novita plant (I) infected with *Micrococcus aloverae* 7 dpi (J) 14 dpi and (K) 21 dpi.

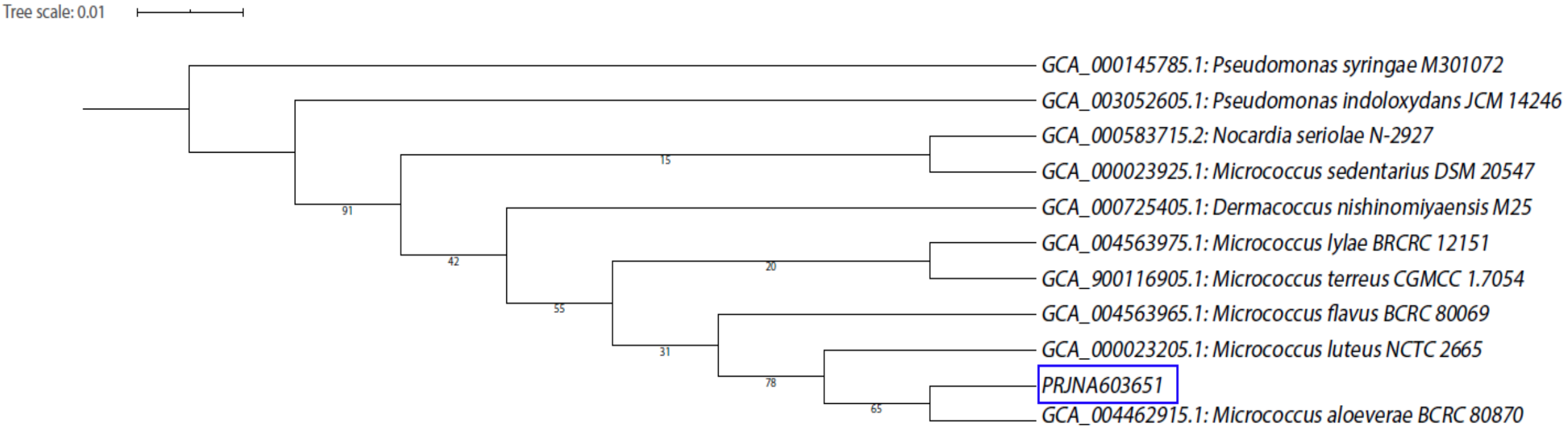

Figure S2. Phylogenetic tree using the whole genome sequencing of the isolated Micrococcus (box, PRJNA603651). Species are shown with accession numbers followed by strain information. *Pseudomonas syringae* was used as an outgroup. RAxML was used to construct a maximum-likelihood phylogeny with the GTRGAMMA substitution model (v8.2.4) (Stamatakis, 2014). The number of seeds for parsimony inferences and rapid bootstrap analysis was set to 12345 (-p 12345 -x 12345, respectively). The robustness of the inferred phylogeny was assessed by 1000 rapid bootstrap (-#1000).