macroscopic morphological traits were reproducible: the correlations between the first diameter measure and the two replicate measures were indeed highly significant (S5 Table; $r = 0.88$, $P < 0.001$ for second replicate and $r = 0.84$, $P < 0.001$ for third replicate) and the same colors, texture and margins were recorded on the different subcultures of the same isolate. Microscopic morphological variations were also observed on MEA medium: conidiophore roughness was more or less pronounced and penicilli were more or less appressed depending on the specimen observed. However, these differences were subtle and were therefore not recorded. No substantial spore size variation was noticed among isolates. Noteworthy, only rudimentary penicilli were observed for isolate MUCL 18048.

Phylogenetic reconstruction

Out of the eleven tested DNA fragments, six were not kept for further analyses (18S, 28S, ITS, *EF-1α*, *rpb1*, *rpb2*) due to their lack of genetic variability on the 24 selected isolates. The five fragments chosen for further sequencing (β-*tub*, *cmd*, *cct8*, *tsr1* and *mcm7*) were successfully amplified by PCR from total DNA extracts of 143 to 145 *P. roqueforti* isolates, depending on the fragments, as well as of 14 strains belonging to other *Penicillium* species. Overall, the partial *β-tub* locus (443 bp), *cct8* locus (1224 bp), *cmd* locus (465 bp), *mcm7* locus (565 bp) and *tsr1* locus (809 bp) were used to construct five gene genealogies (Fig 2; Table 3).

Due to the weak phylogenetic signal of the first five genes (Table 4), three more polymorphic DNA fragments (*Proq235*, 930 bp; *Proq631*, 1029 bp; *Proq845*, 988 bp) were identified by comparing five *P. roqueforti* strains genome sequences and sequenced on the 30 isolates chosen for further phylogenetic reconstructions (S1 Fig; Table 3).

Because the ILD tests indicated significant incongruence between the studied gene trees in both datasets (β-*tub*, *cmd*, *cct8*, *tsr1*, *mcm7* on the one hand and *Proq235*, *Proq631*, *Proq845* on the other hand) ($P < 0.05$), no phylogenetic analyses were performed using concatenated datasets.

Species delimitation

Considering the five individual gene trees for *ßtub*, *cct8*, *cmd*, *mcm7* and *tsr1*, isolates identified as *P. paneum*, *P. carneum* and *P. roqueforti* were systematically assigned to three distinct clades regardless of the method used (ML, MP or Bayesian inference). In contrast, *P. psychrosexualis* isolate CBS 128137^{HT} appeared as a sister clade of *P. roqueforti* in the *cct8* tree, as a sister clade of *P. carneum* in the *ßtub* tree and basal to *P. carneum* and *P. roqueforti* according to the *cmd* tree and even within the *P. roqueforti* clade in the case of the *mcm7* and *tsr1* trees. In each of the five gene genealogies, multiple subclades appeared within the *P. roqueforti* clade. Some of these subclades were well supported (*e.g.*, in the *ßtub* and *tsr1* trees) but not others (*e.g.*, in the *cmd* tree). When considering the well-supported subclades, they did not consistently include the same isolates across the different gene trees. For example, the FM164 and CBS 498.73 isolates were nested in the same subclade in the *ßtub* genealogy while placed in two different subclades in the *tsr1* genealogy.

Regarding the individual gene trees obtained for the *Proq235*, *Proq631* and *Proq845* variable regions using only *P. roqueforti* isolates ($n = 30$), all subclades were well supported but again conflicts were observed between the different gene genealogies with regards to *P. roqueforti* subclades relationships and content. Incongruences among the nodes between the different gene genealogies in *P. roqueforti* were observed, as illustrated on the cluster network consensus tree (Fig 3), indicating relatively recent recombination among these groups. No cryptic species could therefore be recognized according to the GC-PSR method.