genealogies, because recombination leads to their incongruence. The GC-PSR criterion thus only applies to sexual species. *P. roqueforti* has recently been shown to be able to undergo sex and recombination footprints and indirect evidence of recent sex in populations have been observed [16,20]. The GC-PSR criterion is however conservative: it will not distinguish recently derived species in which coalescence of alleles is not achieved yet [17,21–23]. Therefore, more rapidly evolving markers (microsatellites) were also developed, using the recently published genome sequence of *P. roqueforti* FM164 [4]. Furthermore, the morphological variability in our *P. roqueforti* collection was assessed. The goal of this study was to assess whether different cheese-making processes have used or generated different genotypes or cryptic species within *P. roqueforti*.

Materials and Methods

Penicillium roqueforti collection

A *P. roqueforti* collection was established by isolating strains from 120 individual blue-veined cheeses (of either artisanal or commercial origin), collected from 18 different countries worldwide (Argentina, Brazil, Canada, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Latvia, The Netherlands, New Zealand, Poland, Spain, Switzerland, United-Kingdom and the USA). Information about the cheeses sampled is given in S1 Table. For each cheese, six samples were plated in order to obtain six distinct isolates per cheese. The characterization of each isolate was performed using morphological and ß-tubulin partial gene sequence as described below. For each sampled cheese, a single isolate representative of each morphological type observed was eventually kept in the working collection. In total, 164 *P. roqueforti* isolates were available for this study including 27 *P. roqueforti* isolates from 21 different non-cheese substrates (silage, fruit, bread, meat, human sputum and cork) obtained from culture collections. In addition, 14 strains belonging to other terverticillate *Penicillium* species were used in order to assess relationships within the section *Roquefortorum* (S2 Table).

Morphological observations and statistical analyses

Macroscopic colony morphology (color obverse; texture; diameter and margin) of the 164 isolates were observed on PDA medium (Potato Dextrose Agar, Difco, Becton Dickinson and Company) after 7 days incubation at 25°C. Color obverses were assigned to each isolate using the *Munsell Soil Color Charts* [24]. Three sub-cultures on Potato Dextrose Agar (PDA) (25°C) and also on Czapek Yeast Extract Agar (CYA) (5°C, 25°C & 37°C), Glycerol Nitrate Agar (G25N) (25°C) and Malt Extract Agar (MEA) (25°C) media for 7 days as described by Pitt [25], were done for the most distinguishable morphological types. Regarding macroscopic morphology, reproducibility was checked using three sub-cultures of a subset of 36 isolates. Statistical tests on morphologies were performed using JMP version 7 [26]. Microscopic morphology was also investigated by observing specimens sampled from the subcultures on MEA medium.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from fresh mycelium for each isolate after 5–7 days growth on M_2Lev (20 $g.L^{-1}$ malt extract, 3 $g.L^{-1}$ yeast extract and 15 $g.L^{-1}$ agar) using the FastDNA SPIN Kit (MP Biomedicals, Illkirch, France) according to the manufacturer's instructions. Stock solutions (100 $ng.\mu L^{-1}$) were prepared for PCR experiments and all DNA samples were conserved at -20°C.