INTRODUCTION

Cold-adapted fungi are ubiquitous in cold habitats such as the deep seas, Arctic and Antarctic areas, and glaciers. Cold-adapted fungi have evolved special properties, e.g., cold adapted enzymes, change of membrane fluidity, and other cellular components, to enable them to grow at low temperatures at rates comparable to those of mesophiles at moderate temperatures (D’Amico et al. 2006, Ruisi et al. 2007). During the past two decades, research on cold-adapted fungi has increased, driven by their potential value for application in biotechnology (Margesin & Schinner 1994, 1999). Cold-adapted fungi have become important sources for the discovery of novel bioactive secondary metabolites and enzymes (Flam 1994, Pietra 1997, Biabini & Laatch 1998, Gudjarnnsson 1999, Höller et al. 2000, Verbiest et al. 2000, Bhadury et al. 2006, Ebel 2006, Blunt et al. 2007, Rateb & Ebel 2011).

Microorganisms living in low temperature environments are generally referred to as psychrophiles or psychrotolerants. Psychrophiles have been defined as species that can grow at or below 0 °C; have optimum growth temperatures (OGT) of ≤ 15 °C and maximum growth temperatures (MGT) of ≤ 20 °C; while psychrotolerants can grow close to 0 °C, have OGT > 15 °C and MGT > 20 °C (Morita 1975). However, these definitions are also ambiguous and may not be applicable for most of the eukaryotes, as some higher organisms known as psychrophiles, such as some algae, plants, insects, marine and terrestrial invertebrates, and fish may have much broader growth-temperature ranges. The terms stenopsychrophile and europsychrophile have therefore been proposed to modify the definitions of psychrophic and psychrotolerant. The ‘steno-’ and ‘eury-’ are referred ecological terms derived from Shelford’s law of tolerance that describe narrow or wide tolerance to an environmental determinant, respectively. The stenopsychrophile (equal to ‘psychrophile’) refers to microorganisms with a restricted growth-temperature range that cannot tolerate higher temperatures. Eurypsychrophile (equal to ‘psychrotolerant microorganisms’) describes microorganisms that ‘like’ permanently cold environments, but can also tolerate a wide range of temperatures extending into the mesophilic range (Cavicchioli 2006).

Since the discovery of bioluminescent bacteria that are able to grow at 0 °C by Forster (1887), a number of psychrophilic bacteria have been discovered from deep ocean sediments, glacier ice, and soils of the polar regions (DeLong et al. 1997, Mountfort et al. 1998, Price 2000, Berestovskaya et al. 2002, Margesin et al. 2003, Bowman et al. 2004, Seo et al. 2005, Zhang et al. 2006, 2008, Grünke et al. 2012). However, the number of known cold-adapted fungi, especially psychrophilic fungi, is relatively low. In recent years, the diversity of filamentous fungi in cold niches has been increasingly investigated, and the number of known species has greatly expanded (Möller & Dreyfuss 1996, Robinson 2001, Blanchette et al. 2004, Arent et al. 2006, Connell et al. 2006, Held et al. 2006, Malosso et al. 2006, Duncan et al. 2008, Onofri et al. 2008, Selbmann et al. 2008, Arentz & Blanchette 2008, Jurgens et al. 2009). Most species in these studies, however, are psychrotolerant, and only a few were documented as psychrophiles such as Thelobolus microporus, Mucor strictus, Phoma herbarum, Humicola marvinii, Pseudogymnoascus destructans, and some snow molds (e.g. Sclerotinia borealis, Microdochium nivale, Coprinus psychromorbidus) (Schipper 1967, Dejardin & Ward 1971, Traquair & Smith 1982, Richard et al. 1997, Hsiang et al. 1999, Tronson et al. 2001, Singh et al. 2006, Gargas et al. 2009, Hoshino et al. 2010, Anupama et al. 2011, Minnis & Lindner 2013). Species in several yeast genera including Mria, Mrikaia and Rhodotorula were usually described as psy-
chrophilic. For example, *Mrikia frigida* grew well at 15 °C and 4 °C but poorly at 20 °C (Margaret 1966); *Mrikia psychrophila* from Antarctic soil had an optimal growth temperature of 10 °C and a MGT of 18 °C (Xin & Zhou 2007); *Mrakkiella cryocotroctii, M. aquatica* and *M. nicombisi* from alpine and arctic habitats also exhibited psychrophilic features and failed to grow at temperatures over 20 °C (Marcosin & Fell 2008, Robin et al. 2010). Psychrophilic fungi are phylogenetically diverse and we identified the cold-adapted fungi through a polyphasic approach integrating phylogenetic analysis, morphological characterisation and cold-adapted features in the present study.

The Qinghai-Tibet Plateau, often called the ‘world’s roof’ or ‘the third pole’, is located in the southwest of China and is the highest and largest low-latitude region with permafrost in the world. The high elevation and low latitude make the Qinghai-Tibet Plateau a unique alpine ecosystem that is sensitive to changes in climate and surface conditions (Cheng 1998). In the last 30 years, the permafrost area on the Qinghai-Tibet Plateau has decreased by over 10 000 km² (Li & Cheng 1999).

Therefore, researchers have been paying more attention to investigate microorganisms on the Qinghai-Tibet Plateau. Although prokaryotes have been extensively investigated in this area (Xiang et al. 2005, 2009, Liu et al. 2006, 2007, 2009a, b, Yao et al. 2006, Zhang et al. 2007, 2009, Yang et al. 2008), fungi have not received much attention. During an investigation of the cold-adapted fungi of the Qinghai-Tibet Plateau, 1 428 fungal isolates were obtained, of which 150 species were preliminarily identified. In this paper, we studied some dominant fungi from Qinghai-Tibet Plateau in Helotiales in detail. A few related isolates from the Antarctic were also included.

**MATERIALS AND METHODS**

**Sample collection**

Soil samples were collected from seven glaciers in 2009–2011. The sampling areas were located at the edge or centre of the following glaciers: Midui and Zhadang Glacier in Tibet, Qiay and Toumingengke Glacier in Gansu Province, Hailuogou Glacier in Sichuan Province, Yuzhufeng Glacier in Qinghai Province and Mingyang Glacier in Yunnan Province. In addition, some soil samples were also collected from Antarctica, near the Great Wall Station in January 2011 (Table 1). For all sampling, clean hand tools were surface sterilized with 70% ethanol before use. After the removal of the top 5–10 cm of surface sediment, c. 500 g soil sample was collected from the underlying layer and placed in a fresh Zip-lock plastic bag. The samples were maintained at 4 °C until arrival at the laboratory.

**Isolation of fungi**

Fungi were isolated from soil samples as soon as they were taken to the lab using a traditional pour plate method. A 10 g quantity of each soil sample was suspended in sterile-distilled water in a flask. The volume was then increased to 100 mL before the suspension was shaken to disperse soil particles and then serially diluted to 10⁻², 10⁻³ and 10⁻⁴. For selection of psychrophilic or psychrotolerant fungi, about 0.1 mL of each dilution was placed on the surface of three 90 mm diam Petri plates containing 1/4 PDA (potato dextrose agar plus chloramphenicol at 0.1 mg/mL and streptomycin at 0.1 mg/mL to suppress bacterial growth) and spread evenly. The plates were sealed and incubated at 4, 10 and 20 °C (one plate per temperature). The plates were examined for fungal growth at 1 wk intervals for 4 wk. Colonies that appeared on the plates were transferred to three new plates, which were incubated at 4, 10 and 20 °C as temperature test. The change in colony diameter after 4 wk (growth rate) was determined for each isolate at the three temperatures. The psychrophilic and psychrotolerant fungi isolated in this study were consolidated but not strictly defined by the definition given by Morita (1975). The fungi grew better at 4 and 10 °C than at 20 °C and those that grew better at 20 °C were considered psychrophilic and psychrotolerant. The ex-type specimens (dried culture) were deposited in HMAS (Herbarium Mycologicum Academia Sinicar), with the living culture in CGMCC (China General Microbiological Culture Collection Center).

**Morphological observations**

A number of psychrophilic or psychrotolerant fungi were isolated. Among them, *Phoma sclerotoides* and *Pseudogymnomascus pannorum (= Geomyces pannorum*) were most frequently encountered (137 and 52 isolates, respectively) and are well-known cold-adapted species. Sixteen isolates representing some frequently encountered fungi (190 isolates in total) in the *Helotiales* were studied in more detail. Morphological characteristics were observed, photographed, and measured using material from agar plate and slide culture (Coetzee & Eicker 1990). The colony diameter of fungi growing on PDA plates was measured in two perpendicular directions after 4 wk at different temperatures, and the mean diameter was obtained from five replicate plates cultivated at the same temperatures. Morphological characteristics of colonies including aerial mycelium, density, and pigment production were noted. Microscopic morphology was examined using slide cultures: each isolate was transferred to a 50 mL centrifugal tube and incubated at 10 °C for 3 wk before hyphae, conidiophores, and conidia on water mounts were observed, photographed, and measured with a Nikon 80i microscope with differential interference contrast (DIC) optics.

**DNA extraction, PCR amplification, sequencing, phylogenetic analysis and SNP detection**

Genomic DNA was extracted from the fungal mycelia on PDA plates following the protocol described by Wang & Zhuang (2004). The primers LROR and LR5 (Vilgalys & Hester 1990) were used to amplify the large subunit nrDNA (LSU); ITS1 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer region of the nuclear ribosomal RNA gene; EF1-728F and EF1-986R (Carbone & Kohn 1999) were used to amplify partial translation elongation factor 1-α gene (TEF1), and Bt-2a and Bt-2b (Glass & Donaldson 1995) were used to amplify partial β-tubulin gene (TUB). PCR was
| Species                        | Strain number | LSU            | ITS            | TEF1         | TUB            |
|-------------------------------|---------------|----------------|----------------|--------------|----------------|
| Arachnopeziza varieteplosa    | M337          | –              | –              | –            | FJ477045       |
| Ascoscyoreae sarcoides        | OSC #100772   | FJ176886       | –              | –            | –              |
|                                | –             | AJ406399       | –              | –            | –              |
| Botrytis fuckeliana           | LGM002        | –              | KC683713       | –            | KC683712       |
| Bulgaria inquinans            | ZW–Geo52–Clark| AY789344       | –              | –            | –              |
|                                | CBS 118.31    | DQ470960       | –              | –            | –              |
| Cadophora fastigiata          | DAOM 225754   | JN838877       | –              | –            | –              |
| Cadophora luteo-olivacea      | Glo-40        | –              | HQ661093       | HQ661078     | HQ661063       |
| Catenillula luxurians          | CBS 647.75    | –              | GU727560       | GU727569     | –              |
| Ciboria camelliae             | EFA 1         | –              | FJ959095       | GQ181121     | –              |
| Cistella spicicola            | CBS 731.97    | –              | GU727553       | GU727565     | –              |
| Cudoniella clavus             | OSC 100054    | DQ470944       | –              | –            | –              |
|                                | ILLS0488      | JN012006       | –              | –            | –              |
| Cudoniella indica             | VG 113-4      | GQ477325       | –              | –            | –              |
|                                | VG 112-1      | GQ477324       | –              | –            | –              |
| Cudoniella tenuispora         | ILLS0490      | JN012008       | –              | –            | –              |
| Dermea acerina                | CBS 161.38    | DQ247801       | –              | –            | –              |
| Fabrella tsaqae               | –             | AF366994       | –              | –            | –              |
| Hyaloscypha aureliella         | M235          | EU940153       | –              | –            | –              |
|                                | M234          | EU940152       | –              | –            | –              |
| Hyaloscypha daedaleae         | ZW-Geo138-Clark| AY789415       | –              | –            | –              |
| Hyaloscypha fuckelii          | M233          | EU940154       | –              | –            | –              |
| Hyaloscypha hepaticola        | M339          | EU940150       | EU940226       | –            | –              |
| Hyaloscypha vitreola          | M236          | EU940156       | –              | –            | –              |
|                                | M39           | EU940155       | –              | –            | –              |
| Hymenoscyphus pseudoosbiidius | FC-2799       | –              | AB705220       | AB705213     | –              |
| Hyphodiscus hynienophilius    | CBS 529.87    | GU727555       | –              | –            | –              |
|                                | CBS 602.77    | –              | DQ227264       | DQ227270     | –              |
| Leotia lubrica                | OSC 10001     | NG 027596      | –              | –            | –              |
|                                | ZW-Geo59-Clark| AY789359       | –              | –            | –              |
| Loramycyes macrosporus        | CBS 235.53    | DQ470957       | –              | –            | –              |
| Neofabraea perennans          | RGR 90.0107   | –              | AF281397       | AF281476     | –              |
| Phacidium lacerum             | CBS 130.30    | DQ470976       | –              | –            | –              |
| Phialocephala fortii         | K03 385       | –              | –              | –            | DQ274568       |
| Psilocybe cyanescens           | –             | –              | –              | –            | DQ274834       |
| Pleurotus ostreatus           | CBS 341.62    | DQ70963        | –              | –            | –              |
| Sclerotinia sclerotiorum      | CBS 499.50    | AF431951       | –              | –            | –              |
|                                | CBS 499.50    | DQ470965       | –              | –            | –              |
|                                | WZ0007        | AY789347       | –              | –            | –              |
| Tetracladium apineae          | CCM F-23199   | EU883420       | –              | –            | –              |
|                                | CCM F-23399   | EU883421       | –              | –            | –              |
| Tetracladium brevipes         | CCM F-10501   | EU883418       | –              | –            | –              |
| Tetracladium ellipsoides       | MIDU20        | KF768465       | JX001628       | KF768425     | KF768437       |
|                                | MIDU21        | KF768466       | JX001640       | KF768424     | KF768437       |
|                                | MIDU30        | KF768467       | JX001639       | KF768423     | KF768436       |
| Tetracladium furcatum          | CCM F-06983   | KF768456       | JX001638       | KF768421     | KF768441       |
|                                | CCM F-11983   | KF768455       | JX001615       | KF768422     | KF768439       |
| Tetracladium globosum          | HAILU2015     | KF768460       | JX001609       | KF768433     | KF768448       |
|                                | MY24          | KF768461       | JX001618       | KF768427     | KF768443       |
|                                | MY25          | KF768462       | JX001633       | KF768426     | KF768442       |
| Tetracladium marchalianum      | CCM F-26399   | EU883415       | –              | –            | –              |
|                                | CCM F-11391   | EU883417       | –              | –            | –              |
|                                | CCM F-19399   | EU883423       | –              | –            | –              |
| Tetracladium maculiforme      | CCM F-529     | EU883429       | –              | –            | –              |
|                                | CCM F-13186   | EU883430       | –              | –            | –              |
| Tetracladium palmatum         | CCM F-10001   | EU883424       | –              | –            | –              |
| Tetracladium psychrophilum     | HAILU2030     | KF768464       | JX001919       | KF768429     | KF768446       |
|                                | MY376         | KF768463       | JX001929       | KF768428     | KF768447       |
| Tetracladium setigerum        | CCM F-19499   | EU883426       | –              | –            | –              |
|                                | CCM F-20987   | EU883425       | –              | –            | –              |
| Trichoglossum hirsutum         | OSC61726      | AY789313       | –              | –            | –              |
| V amphiprionae flavovirens    | MBH39316      | AY789426       | –              | –            | –              |
| Vibrissea truncorum           | CBS #258.91   | FJ176874       | –              | –            | –              |
|                                | CUP 62625     | AY789402       | –              | –            | –              |

1 LSU: large subunit nrDNA; ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; TEF1: partial translation elongation factor 1-alpha gene; TUB: partial beta-tubulin gene.
performed in 25 μL reactions containing DNA template 1.0 μL, each forward and reverse primers 1.0 μL, 2 × MasterMix 12.5 μL (TIANGEN Co. Ltd., Beijing, China) and H₂O, using the following parameters: 94 °C for 40 s; followed by 40 cycles at 54 °C for LSU, 53 °C for ITS, 55 °C for TEF1 and 52 °C for TUB gene for 50 s and 72 °C for 60 s; and a final extension at 72 °C for 7 min. The PCR products were sequenced with primers mentioned above by Invitrogen Biotechnology Co. Ltd. (Beijing, China). Sequences were compared to accessions in the GenBank database via BLASTn searching to find the most likely taxonomic designation (Table 2).

Sequence data of the four genes were aligned with Clustal X (Thompson et al. 1997). Further manual alignment was carried out with MEGA v. 5 (Tamura et al. 2011) and alignments were deposited in TreeBASE (www.treebase.org, submission no. S16864). Maximum Parsimony (MP) analyses were conducted using PAUP v. 4.0b10 (Swofford 2002) and Bayesian analysis using MrBayes v. 3.1.2 (Altekar et al. 2004). For the MP analysis, ambiguously aligned regions were excluded from all analyses. An unweighted parsimony (UP) analysis was performed. Trees were inferred using the heuristic search option with TBR branch swapping and 1 000 random sequence addi-
Branches of zero length were collapsed and all equally most parsimonious trees were saved. Descriptive tree statistics such as tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI), were calculated for trees generated. Clade stability was assessed using bootstrap analysis with 1 000 replicates, each with 10 replicates of random stepwise addition of taxa. For the Bayesian analyses, the models of evolution were estimated by using MrModeltest v. 2.3 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC). Six simultaneous Markov chains were run for 1 000 000 generations and trees were sampled every 100th generation (resulting in 10 000 total trees). The first 2 000 trees represented the burn-in phase of the analyses and were discarded and the remaining 8 000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. Trees were visualised in TreeView v. 1.6.6 (Page 1996).

Unique fixed nucleotide positions are used to characterise and describe several sterile species (see applicable species notes). For the sterile species that was described, the closest phylogenetic neighbour(s) were selected from Fig. 3 and 4, and this focused dataset was subjected to SNP analyses. These single nucleotide polymorphisms (SNPs) were determined for each aligned data partition using DnaSP v. 5.00.07 (Librado & Rozas 2009).

**Fig. 2** Phylogenetic tree derived from maximum parsimony analysis based on LSU rDNA sequences (TL = 325, CI = 0.7815, RI = 0.9015, HI = 0.2185 and RC = 0.7046). *Trichoglossum hirsutum* OSC61726 was used as outgroup. The LSU alignment consists of 845 characters, with 151 phylogenetically informative positions. Bootstrap values of more than 50 % are shown on the respective branches and significant Bayesian posterior probability (≥ 95 %) are indicated as **bold** branches. Ex-type cultures are marked with asterisks (*).
RESULTS

In the current investigation, 1,428 fungal isolates were obtained from 350 samples, which were mainly collected from seven glaciers on the Qinghai-Tibet Plateau; a few specimens were collected from Antarctica. Isolates were preliminarily identified to belong to 78 genera representing 150 species. About one-tenth of these isolates were psychrophilic (stenospsychrophilic), mostly belonging to the genera Pseudogymnoascus, Phoma, Tetradiadium and Psychrophila, the new genus described in this paper. Based on the preliminary identification, 16 isolates belonging to Helotiales, which were the most frequently encountered cold-adapted fungi, were selected to study in more detail.

**Phylogenetic analysis and SNP detection**

The phylogenetic relationships were determined for 16 isolates. According to the phylogenetic trees based on the partial large subunit nrDNA (LSU), 16 isolates clustered into two independent clades that were well supported and separated from each other known genera in the Helotiales (Fig. 1) and should represent a new genus and the other eight isolates clustered within the Tetradiadium clade (Fig. 2). In the phylogenetic trees (Fig. 3, 4) generated from combined sequences of ITS+TEF1+TUB, the isolates in Fig. 1 clustered into one clade comprising three subclades that were well supported and separated from each other. Based on phylogenetic relationships and morphological characteristics, a new genus, Psychrophila, is proposed to accommodate these three new species (P. antarctica, P. lutea and P. olivacea).

**Taxonomy**

**Psychrophila** M.M. Wang & Xing Z. Liu, gen. nov. — Myco-Bank MB801296

Etymology. *Psychrophila* means cold-loving and is referring to those fungi well adapted to low temperature habitats.

Type species. *Psychrophila antarctica* M.M. Wang & Xing Z. Liu.

Colonies on PDA slow-growing, cream-white, yellowish or dark-olive to dark-brown, with sparse aerial mycelium; vegetative hyphae hyaline, smooth, thick-walled, transversely septate, most agglomerate to bundles, or swollen to moniliform. The cells of aerial hyphae often aggregated in dense clumps, hyphae deep immersed into the agar. *Conidiogenous cells* phialidic, entero-blastic, hyaline, flask-shaped, apically tapering into a broad funnel, bottleneck-like constriction; the collarette wedge-shaped to campanulate and widely flaring. *Conidiophores* reduced to...
conidiogenous cells, sometimes short, or much differentiated. Conidia hyaline, smooth, aseptate, pyriform to globose, within a single conidiogenous locus.

Habitat — Cold environments.

Notes — Species with phialophora-like asexual morphs in the Helotiales include: Ascocoryne with Coryne asexual morphs, which have hyaline, more or less penicillate conidiophores and phialides that lack visible collarettes; asexual morphs of some species of the Dermateaceae, such as Mollisia and Pyrenopeziza, might be accommodated in Cadophora, which has more or less pigmented vegetative hyphae, pale to hyaline phialides and collarettes (Gams 2000); the asexual morph of Hyphodiscus hymenophilius is Catenulifera rhodogena, which has cylindrical to ampulliform phialides, long and cylindrical collarette, and conidia born in chains or in droplets (Hosoya 2002), in contrast, species in the new genus Psychrophila have hyaline vegetative hyphae, phialides, and collarettes; conidiophores are reduced to conidiogenous cells, sometimes short, or much differentiated; collarettes are wedge-shaped to campanulate and widely flaring; and conidia are hyaline, pyriform to globose. The combination of a cold-adapted nature, morphological characters, and phylogenetic relationships well supports the establishment of the new genus Psychrophila (Fig. 1).

**Psychrophila antarctica** M.M. Wang & Xing Z. Liu, sp. nov. — MycoBank MB801298; Fig. 5

*Etymology.* Antarctica refers to the type locality of this fungus.

*Colony* on PDA at 10 °C attaining 25 mm diam after 4 wk, OGT 20 °C, euryspsychophore; colonies cream white, aerial mycelium less abundant or sparse on the surface of the colony. Conidiophores sometimes short, or much differentiated, conidiogenesis phialidic, phialides short, hyaline, flask-shaped, 5.1–8.0 × 2.5–4.5 μm (mean ± S.D. = 6.4 ± 0.89 × 3.5 ± 0.77 μm, n = 30), apically tapering to a broad funnel, bottleneck-like constriction; the collarette 2.1–4 μm (mean ± S.D. = 2.9 ± 0.56 μm, n = 30), wedge-shaped, widely flaring; vegetative hyphae hyaline, sometimes agglomerate to bundles or swollen to irregular shapes, 2–4 μm. Conidia hyaline, 1-celled, smooth, mostly globose, 2.1–3.5 μm diam (mean ± S.D. = 2.7 ± 0.47 μm, n = 30).

Specimen examined. **Antarctic**, Great Wall Station, S62°12’ W58°57’, from soil, Jan. 2011, T. Zhang (dried culture HMAS244374 holotype, living culture ex-type CGMCC315133 (ANT92)).

Other isolates examined. **Antarctic**, Great Wall Station, S62°12’ W58°57’, from soil, Jan. 2011, T. Zhang, living cultures ANT90, ANT94.

Notes — **Psychrophila antarctica** is a psychrotolerant fungus with an OGT of 20 °C. This species is known from both Antarctica and the Qinghai-Tibet Plateau, whose origin and evolution deserve further studies.

**Psychrophila lutea** M.M. Wang & Xing Z. Liu, sp. nov. — MycoBank MB801299; Fig. 6

*Etymology.* Lutea refers to the yellow colour of the colony.

Cultures sterile. **Psychrophila lutea** differs from its closest phylogenetic neighbour, *P. antarctica* (Fig. 3), by unique fixed alleles in three loci based on alignments of the separate loci deposited in TreeBASE as study S16864: ITS positions 76 (A),
316 (C), 413 (C), 416 (A), 449 (T) and 454 (T); TUB positions 134 (T), 138 (A), 147 (A), 170 (A), 206 (C), 294 (T), 303 (C), 305 (G), 314 (T) and 356 (G); TEF1 positions 174 (C), 208 (C), 247 (C), 283 (G), 297 (A) and 327 (A).

Colony on PDA at 10 °C attaining 15 mm diam after 4 wk, OGT 20 °C, eurypsychrophile; bright to brown-yellow, part of the colonies submerged in the medium, hyphae above the medium compacted densely, aerial mycelium absent or sparse, hyaline; vegetative hyphae yellow or brown, smooth-walled, 2–8 μm; aggregated in dense clumps or bundles, sometimes swollen to irregular shapes. Conidiophores and conidia absent.

Specimen examined. CHINA, Sichuan, Hailuogou Glacier, N29°33' E101°58', from soil, 20 Apr. 2011, M. Wang (dried culture HMAS244372 holotype, living ex-type culture CGMCC315134 = HAILUO409).

Other isolates examined. CHINA, Sichuan, Hailuogou Glacier, N29°33' E101°58', from soil, 20 Apr. 2011, M. Wang, living cultures HAILUO374, HAILUO407.

Notes — We have used some low nutrient media such as corn meal agar (CMA) and water agar (WA) to induce strains of *P. lutea* to sporulate without success. Phylogenetic analyses showed that it formed a distinct clade most closely related to *P. antarctica* (Fig. 1, 3) but could be differentiated from the later by SNP analysis.

**Psychrophila olivacea** M.M. Wang & Xing Z. Liu, sp. nov. — MycoBank MB801300; Fig. 7

Etymology. *Olivacea* refers to the olive colour of the colony.

Cultures sterile. *Psychrophila olivacea* differs from its closest phylogenetic neighbour, *P. antarctica* and *P. lutea* (Fig. 3), by unique fixed alleles in three loci based on alignments of the separate loci deposited in TreeBASE as study S16864.

*P. antarctica*: ITS positions 113 (C), 116 (A), 133 (A), 308 (C), 343 (A), 346 (G), 364 (A), 411 (G), 413 (A), 425 (A), 431 (G), 439 (G), 451 (A) and 454 (T); TUB positions 116 (C), 137 (T), 151 (G), 183 (T), 199 (A), 220 (G), 223 (T), 295 (C), 304 (G), 306 (C), 328 (C) and 371 (T); TEF1 positions 174 (C), 195 (G), 203 (A), 208 (C), 247 (C), 252 (C), 297 (A), 306 (T), 307 (T), 308 (G), 313 (A), 327 (C), 333 (T) and 340 (T).

*P. lutea*: ITS position 113 (C), 116 (A), 308 (C), 316 (T), 317 (C), 333 (C), 343 (A), 346 (G), 364 (C), 411 (G), 416 (G), 425 (G), 431 (G), 439 (G), 449 (T), 451 (A) and 454 (T); TUB positions

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**Fig. 5** *Psychrophila antarctica* (from strain ANT92) a. Colony morphology at three temperatures after 4 wk (left-to-right: 4, 10 and 20 °C); b–e. conidiophores and conidiogenous cells; f. conidia. — Scale bars = 10 μm.

**Fig. 6** *Psychrophila lutea* (from strain HAILUO409). a. Colony morphology at three temperatures after 4 wk (left-to-right: 4, 10 and 20 °C); b–e. swollen and aggregated hyphae. — Scale bars = 10 μm.
134 (C), 138 (G), 151 (G), 199 (A), 206 (T), 223 (T), 294 (A), 295 (C), 303 (T), 304 (G), 305 (G), 306 (C), 314 (G), 316 (A), 356 (A) and 371 (T); TEF1 positions 195 (G), 203 (A), 252 (C), 283 (A), 306 (T), 307 (T), 308 (G), 313 (A), 327 (C), 333 (T) and 340 (T).

Colony on PDA at 10 °C attaining 10–15 mm diam after 4 wk, growth rate similar at 10 and 20 °C, stenopsychrophile; light to dark olive, sometimes appearing light grey on the surface because of some young aerial hyphae; part of the colonies immersed in the medium, some hyphae above the medium compact densely, colony surface sometimes furrowed; aerial hyphae sparse, hyaline or olive, vegetative hyphae, olive to dark olive, smooth-walled, 2–7 μm; aggregate in dense clumps or rhizomorphs, sometimes swollen to irregular shapes. Conidio- phores and conidia absent.

Specimen examined. **CHINA,** Sichuan, Haizuo Glacier, N29°33’E101°58’, from soil, 20 Apr. 2011, M. Wang (dried culture HMAS244375 holotype, living culture ex-type CGMCC315135 = HAILUO368).

Other isolate examined. **CHINA,** Sichuan, Haizuo Glacier, N29°33’E101°58’, from soil, 20 Apr. 2011, M. Wang, living culture HAILUO563.

Notes — No conidia or conidiophores were observed for *P. olivacea* on PDA, CMA and WA. *Psychrophila olivacea* differs from *P. lutea* in the colony morphology and OGT.

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**Tetracladium globosum** M.M. Wang & Xing Z. Liu, sp. nov. — MycoBank MB801301; Fig. 8

Etymology. Globosum refers to its globose conidia.

Colonies on PDA at 10 °C attaining 30–45 mm diam after 4 wk, pale yellow to light pinkish, OGT 10 °C, stenopsychrophile; part of the colony immersed in the medium, aerial hyphae sparse and hyaline; vegetative hyphae hyaline, smooth, thin-walled, transversely septate, 1–4 μm. Conidia 1-celled, hyaline, globose, smooth-walled, 3.0–5.5 μm (mean ± S.D. = 4.4 ± 0.81 μm, n = 30), attaching to the hyphae with very short conidio- phores, which are not obvious.

Specimens examined. **CHINA,** Sichuan, Haizuo Glacier, N29°33’E101°58’, from soil, 20 Apr. 2011, Manman Wang, dried culture specimen HMAS244377 holotype, living culture ex-type CGMCC315136 = HAILUO215.

Other isolates examined. **CHINA,** Yunnan, Mingyong Glacier, N28°27’E98°45’, from soil, 4 May 2011, M. Wang, living cultures MY24, MY25.

Notes — Species described in the genus *Tetracladium* are all aquatic and mostly inhabit decaying litter in streams and rivers (Bärlocher 1992). *Tetracladium* species produce tetraradiate conidia, which are thought to aid in their colonisation of substrates (Read et al. 1992). Unlike the previously described species, *T. globosum* has globose conidia, indicating that tetraradiate conidia may be an ecologically adapted characteristic. The OGT
is 10 °C but the fungus can also grow at 20 °C. It is interesting that the OGT of *T. globosum* varied among different isolates. This phenomenon has also been observed in other fungi such as *Pseudogymnoascus pannorum*, indicating psychrophily may be an adapted character (Kochkina et al. 2007).

*Tetracladium ellipsoideum* M.M. Wang & Xing Z. Liu, sp. nov. — MycoBank MB801302; Fig. 9

**Etymology.** *Ellipsoideum* refers to the shape of the conidia.

Colony on PDA at 10 °C attaining 30–40 mm diam after 4 wk, pale to bright yellow, OGT at 10 °C, stenopsychrophile; aerial hyphae absent or sparse; vegetative hyphae hyaline, smooth, thin-walled, transversely septate, 1–3 μm. Conidia borne on short, undifferentiated or sessile pedicels (up to 1 μm long), 1-celled, hyaline, ellipsoid, smooth-walled, 4–6.8 × 2–3.4 μm (mean ± S.D. = 5.3 ± 0.69 × 3.7 ± 0.67 μm, n = 30).

Specimen examined. 〈CHNA〉, Tibet, Midui Glacier, N29°27' E96°30’, from soil, 16 Oct. 2009, Manman Wang (dried culture specimen HMAS244378 holotype, culture ex-type CGMCC315137 = MIDUI20).

Other isolates examined. 〈CHNA〉, Tibet, Midui Glacier, N29°27’ E96°30’, from soil, 16 Oct. 2009, M. Wang, living cultures MIDUI30, MIDUI21.

Notes — The morphology of *T. ellipsoideum* is very similar to that of *T. globosum*. *Tetracladium ellipsoideum* produces conidia that are pyriform to ellipsoid rather than globose as observed for *T. globosum*. Conidiophores are somewhat differentiated and obvious for *T. ellipsoideum* but not obvious for *T. globosum*.

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**Fig. 9** *Tetracladium ellipsoideum* (from strain MIDUI20). a. Colony morphology at three temperatures after 4 wk (left-to-right: 4, 10 and 20 °C); b–d. conidia, conidiogenous cells and hyphae. — Scale bars = 10 μm.

**Fig. 10** *Tetracladium psychrophilum* (from strain HAILUO380). a. Colony morphology at three temperatures after 4 wk (left-to-right: 4, 10 and 20 °C); b–d. hyphae. — Scale bars = 10 μm.
Tetracladium psychrophilum M.M. Wang & Xing Z. Liu, sp. nov. — MycoBank MB801304; Fig. 10

Etymology. Psychrophilum refers to the cold-loving character of the species.

Cultures sterile. *Tetracladium psychrophilum* differs from its closest phylogenetic neighbour, *T. globosum* (Fig. 4), by unique fixed alesis in three loci based on alignments of the separate loci deposited in TreeBASE as study S16864: ITS positions 97 (C), 108 (A), 310 (C), 336 (C), 377 (C), 397 (A), 416 (G), 427 (C) and 430 (G); TUB positions 125 (C), 131 (T), 140 (A), 152 (G), 157 (C), 185 (A), 186 (T) and 196 (C); TEF1 positions 78 (C), 80 (G), 84 (T), 99 (C), 153 (T), 161 (T) and 166 (C).

Colony slow-growing, attaining about 10–15 mm diam on PDA at 10 °C after 4 wk, cream-white to pale yellow; OGT 10 °C, MGT 20 °C, stenopsychrophile; aerial mycelium sparse or absent, conidia absent; vegetative hyphae hyaline, often aggregated, 2–14 μm. *Conidia* and *conidiofores* absent.

*Specimen examined.* CHNA, Sichuan, Halluogou Glacier, N29°33’E101°58’, from soil, 20 Apr. 2011, M. Wang (dried culture HMAS244371 holotype, living ex-type culture CGMCC315139 = HAILUO380).

*Other isolate examined.* CHNA, Yunnan, Mingyong Glacier, N28°27’E98°45’, from soil, 4 May 2011, M. Wang, living culture MY376.

Notes — *Tetracladium psychrophilum* grows slowly with OGT at 10 °C and MGT at 20 °C. Unlike *T. globosum* and *T. ellipsoideum*, *T. psychrophilum* did not produce conidia or conidiofores.

**DISCUSSION**

The rising of the Qinghai-Tibet Plateau was an important geological event in the Quaternary period when the average rate of rising was 1.0–1.1 mm/year. In the last 10,000 years, the plateau has raised 300–700 m and is still rapidly rising (Li & Wang 1983). This steady rising of the Qinghai-Tibet Plateau may have resulted in gradual environmental changes and niches that are inhabited by cold-adapted fungi.

Species of *Geomyces* and *Phoma* are widespread and especially common in northern temperate regions or Arctic and Antarctic permafrost soils. Traditionally, *Geomyces* is characterised by short but distinctly branched conidiophores that have spore attachments to the substrate and provide a stable base for rapid germination (Read et al. 1992). *Tetracladium* species are primary agents of leaf litter and wood decay in streams and rivers. Some aquatic fungi including *Tetracladium* species are distributed worldwide (Descals 1997, Shearer et al. 2007, Wurzbacher et al. 2010) and in lotic habitats from the equator to the Arctic (Shearer et al. 2007). *Tetracladium* species have been documented from streams of alpine glaciers and from snow-covered soil (Robinson et al. 2000, Kuhnt et al. 2012), and are likely to be cold-adapted. The genus is rather homogeneous in terms of cultural characters and conidiogenesis. In addition to the type species, *T. marchalianum*, seven other species have been reported in the genus, e.g. *T. apiense* (Sinclear & Eicker 1981), *T. breve* (Roldán et al. 1989), *T. furcatum* (Descals & Webster 1983), *T. maxilliforme* (Ingold 1942), *T. nitalense* (Sati et al. 2009), *T. palmatum* (Roldán et al. 1989) and *T. setigerum* (Ingold 1942). Recent phylogenetic analyses suggested that the genus is monophyletic and affiliated with Helotiales (Nikolcheva & Bärlocher 2002, Baschien et al. 2006, Letourneau et al. 2010, Seena et al. 2010). The three new species described here are paraphyletically clustered with aquatic *Tetracladium* species. *Tetracladium globosum* and *T. ellipsoideum* produce simple globose or clavate conidia on very short conidiophores or on the hypha (sesill), and *T. psychrophilum* does not produce conidia. All three are clearly different from previously described aquatic species in this genus and may reflect their adaptation to glacial niches with little free water. The three new species can grow well at temperatures below 20 °C and produce colonies that are light or bright yellow or light pink. Their colonies are often flat, with sparse or no aerial mycelium, which may be beneficial for cold-adaptation.

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