A new subclade of haplontic *Metschnikowia* species associated with insects of morning glory flowers in Africa and description of *Metschnikowia aberdeeniae* sp. nov.

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The novel species *Metschnikowia aberdeeniae* is described to accommodate five isolates recovered from insects of morning glory flowers in the Serengeti National Park, Tanzania. On the basis of rDNA ITS and D1/D2 large-subunit sequences, these yeasts form, together with six other isolates, a novel subclade of large-spored *Metschnikowia* species. The exact position of the subclade within the Metschnikowiaceae cannot be determined with any confidence from these sequences or from small-subunit rDNA sequences, as the variable sites of the sequences are excessively divergent. However, in morphological and physiological terms, the novel isolates are typical of the genus *Metschnikowia* in general and of the large-spored group in particular. The type strain of *Metschnikowia aberdeeniae* sp. nov. is strain SUB 05-213.1^T (= CBS 10289^T = NRRL Y-27921^T) (h⁻) and the allotype is strain SUB 05-213.2 (= CBS 10290 = NRRL Y-27922) (h⁺).

Nitidulid beetles and other insects that visit ephemeral flowers harbour a highly specific yeast biota that includes, among others, a group of haplontic, heterothallic *Metschnikowia* species that form unusually large asci (Lachance *et al.*, 2001). These species are unusual also in that their rDNA sequence divergence is nearly as extensive as that found across the entire ascomycetous yeast domain. This is in sharp contrast with the high degree of homogeneity in terms of morphology, life cycle and nutritional attributes of the species. These yeasts show distinct biogeography. Some species are restricted in distribution to the Nearctic region, Central America, Brazil, Hawaii or the Australia–South Pacific region (e.g. Lachance *et al.*, 2005). The distribution of the related asexual species *Candida ipomoeae* is more or less Neotropical. Its range extends from Brazil to Tennessee but does not include the Great Lakes region. The sampling range was recently extended to the African continent. Eight sites in or near the Serengeti National Park, Tanzania, featured stands of *Ipomoea hildebrandtii* (Convolvulaceae), an equatorial African native morning glory. Among the yeasts recovered from insects collected in the flowers were 11 isolates that constitute a novel subclade of the genus *Metschnikowia*. The yeasts are typical of others recovered in similar habitats, but their sequence divergence would suggest a distant ancestry within the Metschnikowiaceae. One taxon, represented by multiple strains of both mating

types, is described as a novel species of the genus *Metschnikowia*, for which the name *Metschnikowia aberdeeniae* sp. nov. is proposed.

Isolation, characterization and ecology

The locations of the collection sites are detailed in Fig. 1 and Table 1. A total of 36 insects, collected in 2004 and 2005, were allowed to walk on the surfaces of plates of YM agar supplemented with 100 mg chloramphenicol l⁻¹. Three different insects were sampled (Table 2). The blister beetle *Mylabris tristigma* (Coleoptera: Meloidae) is a pollen and flower feeder regarded as an important pest of plant crops in sub-Saharan Africa (Abate & Ampofo, 1996). An unidentified buprestid beetle (also known to cause flower damage) and an unidentified *Scaptodrosophila* species also yielded yeasts. Five of the eight collection sites yielded isolates that were kept for further analysis. The yeasts were characterized morphologically and physiologically by using standard methods (Yarrow, 1998). At least one representative of each morphotype of ascomycetous yeast was also identified by PCR amplification and rDNA sequencing, as described previously (Lachance *et al.*, 2005). Mating reactions among *Metschnikowia* strains were obtained by mixing actively growing cells in pairs on yeast carbon base agar supplemented with 0.01% ammonium sulphate. Asci were detected by scanning the plates directly at low magnification after 1 day. The presence of ascospores was assessed after 2–3 days in material mounted on a slide, at higher magnifications. Strains were accessioned in the culture

The GenBank/EMBL/DDBJ accession number for the sequence of the rDNA segment encompassing most of the small subunit and most of the large subunit of strain SUB 05-213.1^T is DQ266429.

Fig. 1. Collection sites in the Serengeti National Park. Sites from which yeasts in the novel *Metschnikowia* subclade were recovered are shown as solid circles; other sampling sites are shown as open circles. Details of sites and samples are given in Table 1. The National Park is outlined in bold.

collection of the Department of Biology, Syracuse University, Syracuse, NY, USA. They are preserved also in liquid nitrogen in the UWOPS yeast collection, Department of Biology, University of Western Ontario, London, Ontario, Canada.

Table 1. Collection sites in the Serengeti National Park

Sample numbers correspond to individual insects.

Site	Reference	Samples	UTM coordinates
locality			
1	Naryswega	04-301 to 04-304	0688446 E 9746524 N
2	Mbuzi Mawe	04-305 to 04-308	0719383 E 9755819 N
3	Mugumu	04-309 to 04-312	0693715 E 9792015 N
4	Massai Kopjes	04-313 to 04-315	0709761 E 9723590 N
5	Seronera Lodge	04-316 to 04-319	0700983 E 9729357 N
6	Musabi	05-201 to 05-203	0681659 E 9747167 N
7	Banagi	05-204 to 05-210	0704223 E 9744339 N
8	Central Hills	05-211 to 05-217	0715782 E 9749437 N

Table 2. Selected properties of *Metschnikowia* isolates from the Serengeti

Strain accession numbers are those in the yeast culture collection of the Department of Biology, Syracuse University, Syracuse, NY, USA (SUB), the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS), and the ARS-USDA Culture Collection, Peoria, IL, USA (NRRL). Mating reactions: sterile, asci; 1, single-spored asci; 2, two-spored asci. ^T, Holotype; ^A, allotype.

Strain	rDNA sequence type	Species assignment	Mating type	Mating with SUB 05-213.1 ^T	Collection site	Insect
SUB 04-304.1	A	<i>Metschnikowia</i> sp.	h ⁺	Sterile, 1	1	<i>Mylabris tristigma</i>
SUB 04-306.1	A	<i>Metschnikowia</i> sp.	h ⁺	Sterile, 1	2	Buprestid
SUB 04-307.1 (=CBS 10291=NRRL Y-27923)	B	<i>Metschnikowia</i> sp.	h ⁺	Sterile, 1, 2	2	Buprestid
SUB 04-310.1 (=CBS 10292=NRRL Y-27924)	C	<i>Metschnikowia</i> sp.	h ⁺	Sterile, 1	3	<i>Mylabris tristigma</i>
SUB 04-311.1	B	<i>Metschnikowia</i> sp.	h ⁺	Sterile, 1	3	<i>Mylabris tristigma</i>
SUB 04-318.1	B	<i>Metschnikowia</i> sp.	h ⁺	Sterile, 1	5	<i>Mylabris tristigma</i>
SUB 05-212.1	A	<i>M. aberdeeniae</i>	h ⁻	–	8	<i>Scaptodrosophila</i> sp.
SUB 05-213.1 ^T (=CBS 10289 ^T =NRRL Y-27921 ^T)	A	<i>M. aberdeeniae</i>	h ⁻	–	8	<i>Mylabris tristigma</i>
SUB 05-213.2 ^A (=CBS 10290=NRRL Y-27922)	A	<i>M. aberdeeniae</i>	h ⁺	2	8	<i>Mylabris tristigma</i>
SUB 05-215.1	A	<i>M. aberdeeniae</i>	h ⁺	2	8	Buprestid
SUB 05-217.1a	A	<i>M. aberdeeniae</i>	h ⁺	2	8	<i>Scaptodrosophila</i> sp.

Largely for reasons of logistics, a detailed quantitative survey of the yeast community of the flowers and their insects was not performed. However, *Metschnikowia* was clearly the dominant genus recovered in these collections. The ascomycetous yeasts *Kodamaea kakaduensis* and *Candida restin-gae*, both of which are typical of ephemeral flower insects (Lachance *et al.*, 2001), were each recovered twice. Three single isolates were identified as *Candida parapsilosis*, a *Candida glabrata*-like species and an unknown *Williopsis* species. Other incidental yeasts included representatives of the genera *Cryptococcus*, *Rhodotorula* and other airborne fungi such as *Aureobasidium*, none of which was identified to the species level.

Two of the 11 isolates were of mating type h^- . Assignment was based on the observation of conjugation tubes (not shown) in mixtures with the type (h^+) of *Metschnikowia continentalis* (UFMG 96-173^T). The tubes reached 40 μ m in length, but adhesion was not observed. None of the isolates, regardless of mating type, reacted in a similar manner when mixed with the allotype of *M. continentalis* (h^- , UFMG 96-179). Mating in all compatible pairs of African isolates gave rise to asci that varied in abundance and sporulation intensity. The five strains recovered from site 8 in 2005 (to be included in *M. aberdeeniae*) exhibited full interfertility, as demonstrated by the formation of abundant two-spored asci (Table 2, Fig. 2a–c); both h^- strains were among these. Crosses between these and the remaining (2004) strains gave rise to fewer asci that were most frequently sterile (Fig. 2f) or single-spored (Fig. 2e, g). Rare two-spored asci (Fig. 2d) were detected in a cross with strain SUB 04-307.1. These interfertility patterns are not entirely consistent with the relatedness inferred from rDNA sequence divergence. The strains collected in 2005 had ITS-D1/D2 sequences identical to those of strains SUB 04-304.1 and SUB 04-306.1, in spite of their apparently lower cross-fertility. Three other strains differed by three substitutions (one in the ITS2 sequence and two in the D1 domain). Strain SUB 04-310.1 exhibited a high degree (8.6 %) of sequence divergence from the rest. The differences (substitutions and indels) were spread across the ITS1, ITS2, D1 and D2 regions. The formation of single-spored asci in crosses involving that strain is therefore surprising.

The prudent conclusion at this time is that only the 2005 isolates should be assigned to a single reproduction-based species. The potential membership of the other strains, including those with identical sequences, will remain uncertain until additional h^- isolates become available. The formation of abundant two-spored asci by every strain, when suitably crossed, would lend considerably more weight to additional species assignments. Although an excellent correlation is normally observed between biological species boundaries and the degree of divergence in rDNA sequences (Kurtzman & Robnett, 1998), exceptions do exist. For example, some large-spored *Metschnikowia* species recently found in endemic beetles of Hawaii (Lachance *et al.*, 2005) could not be delineated strictly on the basis of sequence

Fig. 2. Phase-contrast micrographs of asci of *M. aberdeeniae* strains and related isolates, resulting from crosses between strain SUB 05-213.1^T and strains SUB 05-213.2 (a, b), SUB 05-215.1 (c), SUB 04-307.1 (d), SUB 04-310.1 (e) and SUB 04-318.1 (f, g). Asci were two-spored (a–d), single-spored (e, g) or sterile (f). Bar, 10 μ m.

divergence, although clear reproductive-compatibility groups were identified. The formation of sterile or single-spored asci should not be interpreted as a sign of conspecificity, as shown recently for *Metschnikowia borealis* and *M. continentalis* (Marinoni & Lachance, 2004). Even the occasional formation of two-spored asci is possible in crosses between some sister species of *Metschnikowia* (Lachance & Bowles, 2004). In both of these examples, correct species assignments required the verification of spore viability. In both cases also, it was found that spores that are formed in pairs in the majority of the asci arising in a cross are viable.

Fig. 3. Neighbour-joining phylogram of D1/D2 large-subunit rDNA sequences of selected species of the Metschnikowiaceae. The sequences were chosen so as to represent a broad range of subclades within the group. Bootstrap values of 50% and above are shown (1000 iterations). Branch lengths represent uncorrected distances. Bar, 5% sequence divergence.

The tree presented in Fig. 3 is based on an alignment of the D1/D2 variable domains of the large-subunit rDNA and illustrates the basal position of the African isolates in the clade. The other suggestion arising from the sequence data is that the diversity of flower beetle-associated *Metschnikowia* species in Africa may be just as extensive as that found in the New World or in Hawaii. This inference emerges from the observation that the sequence divergence among the Serengeti isolates exceeds that found among members of the core large-spored subclade (represented here by *M. continentalis* and *Metschnikowia hawaiiensis*) or the *Metschnikowia arizonensis* subclade (represented also by *Metschnikowia dekortorum*). The first subclade contains 10 described species and at least three undescribed species; the second subclade has four described species and two undescribed species.

The rDNA segment encompassing most of the SSU and most of the LSU was sequenced from strain SUB 05-213.1^T (GenBank accession no. DQ266429). Neither coding region supported a convincing placement of the species in one subclade or another in the Metschnikowiaceae. Whereas some regions in the small-subunit rDNA clearly suggested an affinity with the core large-spored group, others supported contradictory kinships. Potentially relevant to the basal status of the novel subclade is the fact that this is the first case in which the principal vectors of morning glory-associated *Metschnikowia* species were not nitidulid beetles. This leaves unanswered the important question of whether the cladogenetic development of these yeasts follows biogeographical lines or alternatively embodies a history of

co-speciation with vector insects. A continued search for nectar-feeding nitidulid beetles in African flowers might provide relevant clues in the future.

The cells and asci (Fig. 1) of *M. aberdeeniae* and relatives are typical of those of the core large-spored species, although the ascus size sits at the lower end of the known spectrum, not unlike that seen in *Metschnikowia hama-kuensis* (Lachance *et al.*, 2005). By contrast, the asci and ascospores of members of the *M. arizonensis* subclade (Lachance & Bowles, 2002, 2004), which is a sister group to the core species, are more divergent morphologically. The formation of very long aseptate tubes, which is widespread in the core large-spored group, was not observed in *M. aberdeeniae*. Likewise, after a full month in Dalmau plate culture, only short chains of submerged cells were formed, to the exclusion of anything that could be construed as pseudohyphal growth, also common in both the large-spored and the *M. arizonensis* clades.

The growth responses of the African isolates were typical of the majority of currently described *Metschnikowia* species, which in general are remarkably similar. In fact, it would be more or less impossible, on the basis of growth profiles alone, to discern the African isolates, not only from most large-spored species, but also from more distant relatives such as *Metschnikowia reukaufii*. Most of the isolates were homogeneous in terms of growth responses, except for some trivial variations in the rates of assimilation of a few carbon compounds such as ethanol, citric acid, gluconic acid and glucono- δ -lactone. Strain SUB 04-310.1 grew more weakly on galactose, β -glucosides, mannitol, glucitol and D-glucosamine as carbon sources, and on lysine as a nitrogen source. Fermentation was also less vigorous in that strain. This sort of variation has been observed, on occasion, in some strains of *Metschnikowia lochheadii*.

Latin diagnosis of *Metschnikowia aberdeeniae* Lachance et Starmer sp. nov.

In medio YM *post dies tres* cellulae singulae, binae, aut in catenis brevis, globosae aut ovoidae (2–3 $\times$ 3–5 μ m). *Cultura in agar*o malti *post dies* 14 *convexa tumulosaque, glabra, candida et butyrosa*. *In agar*o carbonis *fundamento post dies* 14 *pseudomycelium nec mycelium verum non formatur*. *Post dies unus, cellulae stirpium interfertili*um mixtarum *in agar*o carbonis *fundamento tubi junctionis formantur*. *Post dies tres, magni asci cylindrati possunt videri*. *Asci stabiles sunt*. *Ascosporae aculeatae* (1.1–1.5 $\times$ 35–50 μ m). *Glucosum et trehalosum (variabile et exigue) fermentantur*. *Glucosum, sucrosus, galactosus, trehalosus, maltosus, melezitosis, cellobiosus, salicinus, L-sorbusus, D-xylosus, ethanolus (lente et exigue), glycerolus (lente), ribitolus, xylitolus, mannitolus, glucitolus, acidus succinicus (aliquando lente), acidus gluconicus (exigues aut lente), glucono- δ -lactonus (variabile), 2-ketogluconatus, glucosaminus (lente) et N-acetylglucosaminus, at non inulinus, raffinosis, melibiosus, lactosus, methyl α -D-glucosidus, amylosus solubilis, L-rhamnosus, L-arabinosus, D-arabinosus,*

D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, galactitolum, myo-inositolum, acidum lacticum, acidum citricum (aliquando exigue), acetinum, ethyl acetum nec hexadecanum. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 32 °C, at non 34 °C.

Habitat insectos e flores *Ipomoea hildebrandtii* in Hortus Patrius Serengeti, Tanzania. Typus SUB 05-213.1^T (h⁻). Allotypus SUB 04-213.2. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10289^T et CBS 10290 depositae sunt.

Description of *Metschnikowia aberdeeniae* Lachance et Starmer sp. nov.

Metschnikowia aberdeeniae (a.ber.dee'ni.ae. N.L. gen. sing. fem. n. *aberdenniae* of Aberdeen, in honour of Virginia Aberdeen, in grateful recognition of her contributions to yeast ecology).

On YM agar after 3 days at 25 °C, cells are spherical to ovoid, occur singly, in parent–bud pairs or in short chains, and measure 2–3 × 3–5 µm. After 2 weeks, colonies are convex and umbonate, glossy, white and butyrous. In Dalmau plate culture on yeast carbon base agar after 2 weeks, pseudohyphae and true hyphae are not formed. After 1 month, compact chains of undifferentiated, mostly submerged, cells are produced at the edge of the exposed part of the streak culture. On yeast carbon base agar at 18 °C, mixtures of cells of complementary mating types give rise to zygotes and asci after 12–16 h. After 3 days, mature asci containing two acicular ascospores (1.1–1.5 × 35–50 µm) are formed in a persistent ascus that retains conspicuous vestiges of the conjugated parent cells (Fig. 2a–c). Fermentation: in glucose, gas production begins after 2–3 days and a full tube of gas develops after 10 days. With regard to other assimilated sugars, only trehalose is fermented weakly in some strains. Glucose, sucrose, galactose, trehalose, maltose, melezitose, cellobiose, salicin, sorbose, xylose, ethanol (weak or slow), glycerol (slow), ribitol (slow), xylitol, mannitol, glucitol, succinic acid (sometimes slow), gluconic acid (weak to slow), glucono-δ-lactone (variable), 2-ketogluconic acid, D-glucosamine and N-acetyl-D-glucosamine are assimilated, but inulin, raffinose, melibiose, lactose, methyl α-D-glucoside, starch, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, galactitol, inositol, lactic acid, citric acid (sometimes weak), acetone, ethyl acetate and n-hexadecane are not assimilated. Ethylamine, lysine and cadaverine are utilized as nitrogen sources, but sodium nitrate and nitrite are not. Negative for growth in the absence of vitamins. Positive for growth in the absence of amino acids. Positive for growth at 32 °C and negative for growth at 34 °C; growth at 33 °C is variable and weak. Negative for hydrolysis of gelatin and casein. Positive for hydrolysis of Tween 80. Negative for acid

production on chalk agar. Positive for growth in the presence of 10 % NaCl; negative for growth at 15 % NaCl. Growth in the presence of 50 % glucose is slow. Negative for growth in the presence of 10 mg cycloheximide l⁻¹. Positive for growth in the presence of 75 mg cetyltrimethylammonium bromide l⁻¹. Negative for starch production and the diazonium blue B reaction.

The habitat is insects associated with flowers of *Ipomoea hildebrandtii* in the Serengeti National Park, Tanzania. The type strain, strain SUB 05-213.1^T (h⁻), was recovered from a specimen of the meloid beetle *Mylabris tristigma* collected in the Serengeti National Park. The designated allotype is strain SUB 05-213.2 (h⁺), recovered from the same sample. These strains have been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (and the ARS-USDA Culture Collection, Peoria, IL, USA), as strains CBS 10289^T (=NRRL Y-27921^T) and CBS 10290 (=NRRL Y-27922), respectively.

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