Advances in the knowledge of the Inocybe mixtilis group (Inocybaceae, Agaricomycetes), through molecular and morphological studies

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Abstract Inocybe mixtilis constitutes a complex of species characterized by nodulose-angulose spores, absence of cortina and a more or less bulbous marginate stipe that is not darkening when desiccated. In order to elucidate species limits within the I. mixtilis complex, an ITS-RPB2 phylogeny was performed and interpreted using morphological and ecological characters. Six supported clades were obtained in our analyses that correspond to I. mixtilis, I. subtrivialis, and four new species to science: I. ceskae, I. johannis-stangiil, I. nothomixtilis and I. occulta. Species within this complex can be morphologically recognized through a unique combination of morphological characters, such as the spore shape, cystidal length and shape, presence and development of the vellpellis and pleus colour and viscosity. Nevertheless, those characters overlap, especially among I. mixtilis, I. ceskae and I. occulta, and intermediate collections are therefore more reliably identified through ITS-sequencing. Two species, I. ceskae and I. occulta are present in both North America and Europe, while the rest are so far only known in Europe, or Europe and Asia (I. mixtilis). All species, except I. johannis-stangiil, seem to be able to establish ectomycorrhizal association both with conifers and angiosperms. Descriptions, colour illustrations and a key to all known species in the I. mixtilis group are provided.

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INTRODUCTION

Inocybe (Agaricales, Basidiomycota) is one of the most diverse genera of fungi, with around 500 (Kirk et al. 2011) or even considerably more (Matheny 2009) species worldwide and is also one of the most common and abundant in ectomycorrhizal communities. For this reason, Inocybe is one of the most cited genera in mycological and ecological studies of ectomycorrhizal communities and also with a large number of unidentified or misidentified sequences in GenBank (Ryberg et al. 2008). This is due to serious taxonomic and nomenclatural problems that persist in Inocybe, mainly due to the existence of cryptic or semi-cryptic species that can be separated by molecular data, or subtle morphological, ecological or chorological differences. On the other hand, there are many species yet to be described in Europe, especially in the Mediterranean region (Esteve-Raventós et al. 2016).

Molecular data have importantly contributed to infer phylogenetic relationships and to disentangle targeted species complexes within Inocybe. Multigene phylogenetic analyses have shown that Inocybe nests in the Agaricoid clade and is classified in the family Inocybaceae by some authors (Matheny et al. 2006), or in Crepidotaceae when Inocybaceae is merged with its sister family (Petersen et al. 2010). In a study assessing traditional taxonomic schemas through multilocus molecular data, Matheny (2009) discussed phylogenetic relationships within the whole genus. This author placed the genus Inocybe into the more restricted family concept of Inocybaceae. Other approaches for the study of the Inocybaceae were based on other genomic regions like RPB1 and RPB2 (Matheny et al. 2002, Matheny 2005), and even on analysing large sets of unidentified ITS sequences assigned to Inocybe and deposited in public databases (Ryberg et al. 2008). Further studies employing also molecular data have addressed related genera like Aurantella (Matheny & Bougher 2006), subgenera like Malloocybe (Cripps et al. 2010) or sections like Marginatae (Kropp et al. 2010, Esteve-Raventós et al. 2016) or Rimosae (Larsson et al. 2009). At species level, several studies have integrated distributional and/or morphological data together with the ITS region to disentangle species complexes and to describe new species of Inocybe (e.g., Cripps et al. 2010, Larsson et al. 2014, 2017, Esteve-Raventós et al. 2016, La Rosa et al. 2017, Matheny & Bougher 2017). This integrative approach has proved useful to unravel species complexes.

This study targets a species complex that is in need of revision, the I. mixtilis group. Inocybe mixtilis, described by Britzelmayr (1885) and consensually placed in section Marginatae (Kühner 1933), is characterized by its nodulose to angular basidiospores, lacking a cortina, and a more or less bulbous marginate stipe which is not darkening when desiccated. The epitope material proposed for I. mixtilis by Marchetti et al. (2014) allows for a more accurate phylogenetic placement and a stable interpretation of I. mixtilis. Examination and sequencing of further material by us suggest that I. mixtilis is actually a species complex containing several undescribed species. Thus,
Table 1  ITS sequences of the taxa employed in the study, indicating present and original identifications, geographical origin, ecology, voucher numbers, GenBank and UNITE accession numbers. Bold entries refer to newly generated sequences.

| Identification (fl. = flaccid) | Original identification | Geographical origin | Ecology | Voucher specimen | GenBank/UNITE accession number |
|-----------------------------|------------------------|---------------------|---------|-----------------|-------------------------------|
|                            |                        |                     |         |                 |                               |
| Crepidotus calceolus        | –                      | Hungary, Szabolcs-Szatmár-Bereg | Unknown | WU 28902/CRI04A | KF837617 –                   |
|                            | –                      | Finland, Koillismaa  | Betula sp., Picea abies, Pinus sylvestris (holotype) | TUR-A 203414 | KX290789 MH496023 |
| I. ceskei                  | –                      | Finland, Koillismaa  | Betula sp., Picea abies, Pinus sylvestris (holotype) | KX290789 MH496023 |
| I. mixtilis                | Canada, British Columbia | Unknown            | UBC F18984 | KR-M-0043231 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Pseudotsuga menziesii | UBC F1982 | KR-M-0043231 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Unknown            | UBC F19107 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Arbutus menziesii, Pseudotsuga menziesii | UBC F19221 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Arbutus menziesii, Pseudotsuga menziesii | UBC F19221 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Unknown            | UBC F19202 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Unknown            | UBC F18974 | KY933432 –                   |
| I. umbratica               | Canada, British Columbia | Unknown            | UBC F18974 | KY933432 –                   |
| I. praetextiva             | Canada, British Columbia | Unknown            | UBC F18974 | KY933432 –                   |
| I. mixtilis                | USA, Oregon             | Pseudotsuga menziesii | OSC 1084046 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Arbutus menziesii | UBC F19412 | KY933432 –                   |
| I. glabrodisca             | Canada, British Columbia | Unknown            | UBC F19553 | KY933432 –                   |
| I. mixtilis                | Canada, British Columbia | Unknown            | UBC F19553 | KY933432 –                   |
| I. flavobrunnescens        | –                      | Portugal, Estremadura | Quercus faginea | A1 39883 (holotype) | KY933432 |
| I. hirculus                | Finland, Varsinais-Suomi | Betula pubescens, Picea abies, Pinus sylvestris, Salix sp. (holotype) | TUR-A 25771/ K5I | FJS3187 –                   |
| I. johnnisa-stanglii       | Germany, Baden-Württemberg | Betula pendula, Picea omarica (holotype) | KR-M-0038039 | KX290791 MH496018 |
| I. pallida                 | Germany, Oberösterreich | Abies alba, Larix decidua, Picea abies | KR-M-0043320 | KX290791 MH496018 |
| I. pallida                 | Austria, Oberösterreich | Abies alba, Larix decidua, Picea abies | KR-M-0043321 | KX290791 MH496018 |
| I. pallida                 | Germany, Bayern         | Betula sp., Picea omarica | M-0021483 | KX290835 –                   |
| I. kriegsteineri           | Spain, Andalucia        | Pinus pinea | AH 44479 | KJ938770 –                   |
| I. mixtilis                | Germany, Bavaria, Hürbach | Picea abies | M-0219861 (epitype) | KM873869 |
| I. viscosaissima var. bulbosovelata | Italy, Piemonte | Pinus strobus | EF 45/20/00 (paratype of I. viscosaissima var. bulbosovelata) | KX290790 MH496026 |
|                            | Finland, Eletl-Háme     | Betula sp., Picea abies, Pinus sylvestris, Salix sp. | TUR-A 145111 | KX290839 –                   |
|                            | Finland, Eletl-Háme     | Pinus sylvestris, Quercus robur, Tulip sp. | TUR-A 182874 | KX290841 –                   |
| I. tabacina                | Germany, Schleswig-Holstein | Alnus sp., Betula sp. | M-0022249 | KX290836 –                   |
| I. cf. tabacina            | Germany, Baden-Württemberg | In forest | M-002251 | KX290837 –                   |
|                            | Finland, Varsinais-Suomi | Picea abies | TUR-A 1999695 | KX290842 –                   |
|                            | Germany, Sachsen-Anhalt | Picea abies | KR-M-0043295 | KX290794 –                   |
|                            | Germany, Sachsen-Anhalt | Picea abies | KR-M-0043296 | KX290795 –                   |
|                            | Spain, Canary Islands   | Pinus canariensis | KR-M-0043297 | KX290796 –                   |
|                            | Germany, Rheinland-Pfalz | Quercus robur | KR-M-0043298 | KX290797 –                   |
|                            | Germany, Baden-Württemberg | Pinus sylvestris | KL 2604 | KX290837 –                   |
|                            | Germany, Baden-Württemberg | Betula pendula, Pinus mugo, Salix caprea | KR-M-0043300 | KX290799 –                   |
|                            | Austria, Oberösterreich | Abies alba, Larix decidua, Picea abies | KR-M-0043301 | KX290800 –                   |
|                            | Germany, Baden-Württemberg | Picea abies | KR-M-0043302 | KX290801 –                   |
|                            | Germany, Baden-Württemberg | Picea abies | KR-M-0043303 | KX290802 –                   |
|                            | Germany, Rheinland-Pfalz | Alnus glutinosa, Corylus avellana, Pinus sylvestris, Quercus robur | KR-M-0043304 | KX290803 MH496021 |
|                            | Germany, Rheinland-Pfalz | Picea abies | KR-M-0043305 | KX290804 –                   |
|                            | Germany, Rheinland-Pfalz | Picea abies | KR-M-0043306 | KX290805 –                   |
|                            | Austria, Salzburg, Tansweg | Alnus incana, Picea abies | KR-M-0043307 | KX290806 MH496020 |
|                            | Germany, Bayern         | Picea abies | KR-M-0043308 | KX290807 –                   |
|                            | Germany, Baden-Württemberg | Picea abies | KR-M-0043309 | KX290808 –                   |
|                            | Germany, Baden-Württemberg | Picea abies | KR-M-0043310 | KX290809 –                   |
|                            | Germany, Baden-Württemberg | Picea abies | KR-M-0043311 | KX290810 –                   |
|                            | Germany, Hessen         | Picea abies | KR-M-0043325 | KX290811 –                   |
|                            | Spain, Basque Country    | Picea abies | ARAN-Fungi 04711 | MH500842 MH496022 |
|                            | France, Nord Cychingo    | Pseudotsuga menziesii | LIP PAM0503003 | H5566970 –                   |
|                            | Italy                    | Unknown | MCV 2153 | JF906218 –                   |
|                            | Sweden                   | Unknown | EL13104 | AM882838 –                   |
|                            | Sweden                   | Unknown | EL5604 | AM882839 –                   |
|                            | Sweden                   | Unknown | EL2904 | AM882837 –                   |
|                            | Sweden                   | Unknown | EL6904 | AM882835 –                   |
| Identification | Original identification | Geographical origin | Ecology | Voucher specimen | GenBank/UNITE accession number |
|----------------|------------------------|---------------------|---------|-----------------|-------------------------------|
| l. mixtilis (cont.) | Denmark | Unknown | EL16107 |  | FJ550894 – |
| | Estonia, Põlva | Unknown | TU118169 |  | UDRB015355 – |
| | Estonia, Hiiumaa | Unknown | TU118144 |  | UDRB015338 – |
| | Italy | Unknown | MCVE 13860 |  | JF908151 – |
| l. nothomixtilis | Spain, Basque Country | Pinus radiata, Quercus robur | AH 24510 (holotype) |  | XX290780 – |
| | Spain, Balearic Islands, Mallorca | Pinus halepensis | AH 46558 |  | XX290786 MH946025 |
| | Spain, Cataluña | Cistus monspeliensis, C. salviifolius | AH 26921 |  | XX290784 – |
| l. tabacina var. pseudovolvata | France, Île-de-France | Carpinus betulus, Fagus sylvatica, Quercus sp. | PC 0738072 |  | XX290838 – |
| l. mixtilis var. aurata nom. inval. | Italy | Unknown | Herb. Alessio, without number |  | XX290813 – |
| | Italy, Toscana | Quercus sp. | KR-M-0043322 |  | XX290814 – |
| | Germany, Baden-Württemberg | Betula pendula, Fagus sylvatica, Quercus petraea | KR-M-0038133 |  | XX290815 – |
| | Germany, Hessen | Pinus sylvestris, Quercus rubra | KR-M-0043319 |  | XX290816 – |
| l. obtusiuscula | France, Savoie | Salix herbacea | LIP PAM0208170 |  | H956869 – |
| l. ochracea | Germany, Bavaria, Augsburg | Alnus incana, Fagus sylvatica, Salix sp. | M Stangl 88/965 |  | FJ556803 – |
| l. occulta | Spain, La Rioja | Quercus ilex subsp. ballota | AH 36443 (holotype) |  | XX290787 MH946017 |
| | Spain, Castilla-León | Pinus pinea | AH 30977 |  | XX290785 – |
| | Spain, Andalucía | Quercus suber | AH 22108 |  | XX290779 – |
| | Spain, Castilla-La Mancha | Cistus ladanifer, AH 40459 | AH 26978 – |
| | Spain, Castilla-León | Quercus robur | AH 24987 |  | XX290781 MH946016 |
| l. mixtilis | Spain, Cataluña | Salix pyrenaica | AH 21576 |  | XX257359 – |
| | Finland, Enontekiö Lappi | Betula pubescens subsp. czerepanovii | TUR-141565 |  | XX290840 – |
| | Spain, Castilla-León | Fagus sylvatica | AH 24989 |  | XX290782 – |
| | Spain, Cataluña | Dryas octopetala, Salix retusa | AH 26731 |  | XX290783 MH946015 |
| | Germany, Bayern | Larix decidua, Picea abies | KR-M-0038028 |  | XX290817 – |
| | Germany, Rheinland-Pfalz | Picea abies | KR-M-0038125 |  | XX290818 – |
| | Germany, Baden-Württemberg | Hellianthemum nummularium, Pinus sylvestris, Quercus robur | KR-M-0043312 |  | XX290918 – |
| | Austria, Tirol | Picea abies | KR-M-0043313 |  | XX290820 – |
| | Germany, Bayern | Larix decidua, Pinus mugo | KR-M-0043314 |  | XX290821 – |
| | Austria, Kärnten | Picea abies | KR-M-0043315 |  | XX290822 – |
| | Austria, Salzburg, Tamsweg | Picea abies | KR-M-0043316 |  | XX290823 – |
| | Germany, Baden-Württemberg | Picea abies | KR-M-0043323 |  | XX290824 – |
| | Germany, Baden-Württemberg | Fagus sylvatica, Picea abies | KR-M-0043318 |  | XX290825 – |
| | Germany, Baden-Württemberg | Abies procera | KR-M-0043317 |  | XX290826 – |
| | Germany, Rheinland-Pfalz | Betula pendula, Larix decidua, Quercus robur | KR-M-0043230 |  | XX290827 – |
| | Netherlands, Friesland | Pinus sylvestris | KR-M-0038029 |  | XX290828 – |
| | Germany, Bayern | Betula pendula, Larix decidua, Pinus sylvestris | KR-M-0043324 |  | XX290829 – |
| | Finland, Koliismaa | Betula sp., Picea abies, Pinus sylvestris | KR-M-0046554 |  | XX290830 – |
| | Finland, Koliismaa | Betula sp., Picea abies, Pinus sylvestris | KR-M-0046555 |  | XX290831 – |
| | Finland, Koliismaa | Betula sp., Picea abies, Pinus sylvestris | KR-M-0046558 |  | XX290832 – |
| | Finland, Koliismaa | Betula sp., Picea abies, Pinus sylvestris | KR-M-0046557 |  | XX290833 – |
| | Finland, Koliismaa | Betula sp., Picea abies, Pinus sylvestris | KR-M-0046556 |  | XX290834 – |
| l. mixtilis | Estonia, Saare | Unknown | TU120084 |  | UDRB013622 – |
| l. praetervisa | Canada, British Columbia | Unknown | UBC F19174 |  | HQ064492 – |
| l. jacobi | Canada, British Columbia | Mixed forest with Arbutus menziesii and Dodecatheon hendersonii | UBC F19302 |  | HQ064491 – |
| l. mixtilis | Australia, Capital Territory | Unknown | CANB 574539 |  | K9308781 – |
| l. mixtilis | USA, Oregon | Pseudotsuga menziesii | OSC 106411 |  | EU529598 – |
| l. mixtilis | USA, Oregon | Pseudotsuga menziesii | OSC 106412 |  | EU529541 – |
| l. mixtilis | USA, Oregon | Pseudotsuga menziesii | OSC 106415 |  | EU529569 – |
| l. mixtilis | Italy | Unknown | MCVE 5052 |  | JF908121 – |
| l. mixtilis | France, Pas-de-Calais | Betula sp., Pinus pinaster, Populus tremula | LIP PAM 0710104 |  | H9568672 – |
| l. mixtilis | Sweden | Unknown | BJ920916 |  | AM882640 – |
| l. mixtilis | Canada, British Columbia | Unknown | UBC F19341 |  | HQ064490 – |
the aim of this work is to revise the *I. mixtilis* complex based on sequence data of two genetic markers (ITS and RPB2) and morphological data and to contribute to a better understanding of the genus *Inocybe*.

**MATERIALS AND METHODS**

**Morphological study**

Specimens studied were collected across Europe and were mostly deposited in KR (Karlsruhe, Germany), AH (Alcalá de Henares, Spain) and ARAN herbaria (Aranzadi Science Society). Additional collections were loaned from the Johann Stangl (M; Munich, Germany), Jukka Vauras (TUR-A; Turku, Finland) and Guillaume Eyssartier (PC; Paris, France) herbaria, while other collections were loaned from private herbaria: Erminio Ferrari (EF; Italy), Roberto Fernández-Sasas (RFS; Bizkaia, Spain), Jordi Vila García (JVG; Barcelona, Spain) and Agustín Caballero Moreno (AC; La Rioja, Spain).

Microscopical features were described from mountings in water and 3–5% ammonium hydroxide solution and observed by light microscopy with the aid of an oil immersion objective. Photomicrographs were taken with a Nikon (Eclipse 80i) microscope and a digital camera Nikon (DS-5 M) and (in the case of D. Bani

dini) with a Leica DM-750-microscope and a Zeiss AxioCam ERC5s. Scanning electron micrographs were obtained using a Zeiss DSM-950. For ultramicroscopic studies, the samples were dehydrated in aqueous ethanol (70%), dehydrated in concentrated ammonium hydroxide (28–30\% for 30 min, dehydrated in aqueous ethanol (70%) for 30 min, fixed for 2 hours in pure ethylene glycol dimethylether (=1.2-dimethoxy methane), and finally immersed in pure acetone for at least 2 hours. This was followed by critical point drying and sputtering with gold-palladium. This technique allows the use of very little material (small portions of lamellae).

Colors of basidiomata, either in fresh or dry condition, were standardized following Mussett\(^\text{1994}\). Most of the terminology used for microscopic features follows Kuyper (1986) and Vellinga (1988); \(L_{\text{unw}}\) and \(Q_{\text{unw}}\) refer to the mean spore length, width and ratio, respectively. Herbarium acronyms follow Thiers (2012). General bibliography, monographs or contributions to specific groups of *Inocybe* were used in the determination of the samples, i.e., Kauffman (1924), Heim (1931), Malençon & Bertault (1970), Stuntz (1978), Alessio & Rebuffe (1980), Kuyper (1986), Nishida (1989), Stangl (1989), Bon (1998), Kobayashi (2002), Ferrari (2006, 2010), Jacobsson & Larsson (2012), Matheny (2016) and Bandini (2017).

**DNA extraction, amplification and sequencing**

Total DNA from BAN (Bandini) samples was extracted from dry herbarium specimens employing a modified protocol based on Murray & Thompson (1980). A portion of each sample was blended with the aid of a microspatula in 600 \(\mu\)L CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min. at 65 °C. A similar volume of chloroform isooamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. Samples were then centrifuged for 10 min at 13000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70%, centrifuged again for 2 min and dried. Genomic DNA was finally resuspended in 200 \(\mu\)L ddH2O.

For the rest of the specimens, genomic DNA was extracted from dried herbarium specimens using the Preppan Ultra Sample Preparation Reagent (Thermo Scientific, USA). One hundred \(\mu\)L of extraction solution were added to 1.5 ml Eppendorf tubes containing small portions of lamellae and context and vortexed. These tubes were incubated for 10 min at 100 °C in a heated block and then centrifuged at 12000 rpm for 2 min. Fifty \(\mu\)L of the supernatant were transferred to a new tube and stored at -20 °C. DNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).

We sequenced two regions: the nuclear ITS1-5.8S-ITS2 region using the primers ITS1F-ITS4 (White et al. 1990) and the RNA polymerase II subunit (RPB2) between primers RPB2-6F-RPB2-7R for the RPB2 region (Matheny 2005). PCR reactions were performed in 25 \(\mu\)L per reaction under a program consisting of a hot start at 95 °C for 5 (6 in the case of Spanish isolates) min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s, respectively) and a final 72 °C step 10 min. PCR products were checked in 1% agarose gels, and positive reactions sequenced in both directions. Sequencing primers were the same as employed for PCR amplification. Raw sequence data was manually edited using Chromas 2.5.1 software (Technelysium Pty Ltd., Southport, Queensland, Australia). The identity of obtained consensus sequences was verified through the BLASTn query algorithm in GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (http://unite.ut.ee/) databases.

| Identification (i. mixtilis) | Original identification | Geographical origin | Ecology | Voucher specimen | GenBank/UNITE accession number |
|-----------------------------|-------------------------|---------------------|--------|-----------------|-------------------------------|
| *I. occulta* (cont.)        | *I. mixtilis*            | Canada, British Columbia | Unknown | UBC F19030      | HK804488                      |
| *I. mixtilis*               | Italy                    | Unknown             | MCVE 91 | GO-2010-169     | JF908089                      |
| *I. mixtilis*               | Mexico, Morelos          | Unknown             | MCVE 6549 | JF908127     | JF908141.2                   |
| *I. xanthomelas*            | Italy                    | Unknown             | MCVE 9844 | JF908141.2     | JF908141.2                   |
| *I. mixtilis*               | Italy                    | Unknown             | MCVE 9844 | JF908141.2     | JF908141.2                   |
| *I. pheaeostictoides*        | –                        | Spain, Segovia      | *Pinus sylvestris* | AH 9154 (holotype) | KT203789                      |
| *I. praetervisa*            | –                        | Italy, Rabbi        | In coniferous wood | SF229596 (epitype) | KT203792                      |
| *I. praetervisoides*        | –                        | Spain, Madrid       | *Quercus ilex* subsp. *balkota* | AH 29863 (holotype) | KT203794                      |
| *I. salicetica*             | –                        | Finland, Pohjois-Karjala | *Alnus incana, Betula sp.*, *Salix sp.* | TUR-A 19738F/K11 (holotype) | FJ755796                      |
| *I. subtrivalis*            | –                        | Spain, Castilla-La Mancha | *Quercus ilex, Cistus* | AH 19225 (holotype) | MH496013 / MH496014          |
| *I. urbana*                 | –                        | Spain, Madrid       | *Cistus ladanifer, Pinus pinaster* | AH 26789 | KX354977 MH496024 |
| *I. tabacina*               | –                        | France, Wannehin    | *Populus nigra* | LIP PAM05071302 | HQ586865                      |
| *I. xanthomelas*            | –                        | Italy, Torino       | *Tilia sp.* | AMB 17142 (holotype) | KT229613                      |
| *I. xanthomelas*            | –                        | Spain, Madrid       | *Betula pendula* | AH 29895 | KJ939774                      |
Phylogenetic analyses

Two genomic regions were sequenced for phylogenetic reconstruction and two alignments were thus assembled. A first ITS-RPB2 alignment aimed to test species boundaries and the monophyly of the *Inocybe mixtilis* group using representative sequences of each species. This dataset comprised 1410 characters divided into two partitions (ITS, 707 characters; RPB2, 703) and 22 taxa. *Inocybe calopedes* (KJ778839, KJ756415) and *I. subfibrosoides* (KP636879, KM656117) were selected as outgroup because sequences of both markers were available for it in GenBank and blast searches indicated similarity to sequences of the *I. mixtilis* group. Gene congruence was evaluated manually by comparing supported clades among single-gene genealogies (Mason-Gamer & Kellogg 1996). Each locus was subjected to a Maximum Likelihood (ML) analysis as described below. A supported clade for one marker was considered to be in conflict when contradicted with significant support by another (bootstrap support > 70 %). As no conflict was detected, the ITS and RPB2 regions were concatenated. A second ITS alignment was constructed to place specimens sequenced only for the ITS region. This alignment comprised 771 characters and 120 sequences, of which 67 were generated by us, while the rest were downloaded from GenBank and UNITE databases (Table 1). Sequences were aligned in MUSCLE software (Edgar 2004) of MEGA6 (Tamura et al. 2013) and MAFFT (http://mafft.cbrc.jp/alignment/server/; Katoh et al.

![Fig. 1](image_url) The Bayesian 50 % majority-rule consensus tree inferred from ITS-RPB2 regions. Numbers above and below nodes represent Bayesian posterior probabilities and ML bootstrap values, respectively. Only nodes significantly supported in one of the analyses bear values. Sequences of *Inocybe stellata* were used as outgroup. Coloured boxes represent different taxa recognized for the *Inocybe mixtilis* species complex.
Bayesian inference of phylogenetic relationships was performed using MrBayes v. 3.2.3 (Ronquist et al. 2012), applying a Gamma+P-Invvar model of rate heterogeneity and a GTR substitution model. Markov Monte Carlo Chains (MCMC) were run for 2 M generations; two independent runs with four chains were performed for trees sampled every 1000 steps, setting the heating temperature as 0.05. Burnin was set at 25 %. ML phylogenetic analysis was conducted using the dataset alignment with the E-INS-i strategy of MAFFT, employing default settings in RAxML v. 8.2.9 (Stamatakis 2006), via CIPRES platform (https://www.phylo.org/; Miller et al. 2010). To assess branch confidence 1000 non-parametric bootstraps were performed.

RESULTS

The 50 % majority-rule consensus phylogenetic tree obtained in the Bayesian analysis of the ITS-RPB2 alignment is shown in Fig. 1. The inferred tree was fully supported in both analyses of this alignment. The *I. mixtilis* group is monophyletic (PP 1; ML-BP 100 %), being *I. glabrodisca* sister to it. Five of the species attributed here to the *I. mixtilis* group received strong support, namely *I. johannis-stanglii* (PP 1; ML-BP 100 %), *I. mixtilis* (PP 1; ML-BP 100 %), *I. nothomixtilis* (PP 1; ML-BP 100 %), *I. xanthomelas* (PP 1; ML-BP 100 %), *I. phaeocystidiosa* (PP 1; ML-BP 100 %),
I. occulta (PP 1; ML-BP 100 %) and I. subtrivialis (PP 1; ML-BP 100 %), while I. ceskæ was only supported in the Bayesian analysis (PP 0.99; ML-BP 59 %), I. johannis-stanglii and I. subtrivialis form a strongly supported clade that is basal to the rest of the species of the I. mixtilis group (PP 0.98; ML-BP 99 %). 

The 50 % majority-rule consensus tree of the Bayesian analysis of the ITS region shows a similar topology, but I. subtrivialis and I. johannis-stanglii do not form a monophyletic clade (Fig. 2). 

The six species of the I. mixtilis group receive support in at least the Bayesian analysis. Intraspecific sequence variability was extremely low for all treated taxa.

Each of the clades could be morphologically characterized based on a unique combination of several characters, such as the spore shape, the cystidial length, the pileus colour and viscosity, and the presence and development of the velipellis. Nevertheless, these characters were overlapping. Ecology and host specificity showed some patterns (Fig 2). While I. ceskæ and I. subtrivialis are restricted to taiga forests and Mediterranean forests, respectively, other species like I. mixtilis exhibited a broader ecological amplitude and associates with both conifers and angiosperms. Likewise, a species seems to be restricted to Europe (I. mixtilis), while I. occulta and I. ceskæ have a transoceanic distribution and were also present in North America.

TAXONOMY

Inocybe mixtilis (Britzelm.) Sacc., Syll. Fung. 5: 780. 1887. — Fig. 3, 4

Agaricus mixtilis Britzelm., Ber. Naturhist. Vereins Augsburg 28: 152, 1883.

MycoBank number MB141839.

Lectotypus. Britzelmayr (1882), Demirini aus Südbayern: f. 21, sub nomen Agaricus rimosus, designated by Marchetti et al. (2014:149). Epitypus: Germany, Bayern, Hörbach, Haspelmoor, Landkreis Fürstenfeldbruck, Fichtenwald (Picea forest), auf Nadelstreuhumus wachsend, gesellig, 26 Sept. 1981, leg. J. Stangl, M-0219661, designated by Marchetti et al. (2014:149). Epitypus: men Agaricus rimosus 1885.

Selected descriptions and iconography — Stangl (1984), Marchetti et al. (2014: 149).

Basidioles often gregarious, rarely solitary. Pileus 10–35–(45) mm diam, 5–20 mm high; shape when young subconical, paraboloid or campanulate, then hemispherical, broadly convex to expanded, often showing a broad distinct umbo even in very young fruitbodies, leaving delicate appendiculate whitish remnants on the pileus margin (KR-M-0043301), or very unusually (as commented by Ferrari 2006 for I. viscosissima var. bulbosovellata), covered by a thin velar patch (‘bianco-grigiognolo’, greyish white); surface distinctly lustrous and sticky in wet conditions and hence agglutinating soil particles or humus remains. Lamellae subventricose, but almost linear in some collections (e.g., KR-M-0043302), 2–5 mm deep, almost free to adnexed, sometimes also adnate with a decurrent tooth, usually rather crowded, L = 50–60–(70), l = 1–2–(3); colour initially white or whitish, then greyish, quite often showing a faint violet hue, brownish grey, sometimes pale rusty-brown or pinkish rusty-brown when old; edge even to crenulate, whitish or paler than faces, but in old specimens sometimes concolorous or even somewhat darker. Stipe 15–50–(60) × (2–)3–5–(6) mm, solid, fibrose; shape cylindrical or tapering upwards, sometimes more or less curved near the base, which is always distinctly marginately bulbous; bulb 7–12 mm broad, edge mostly sharp or even volviform, occasionally showing a fibrose rim from velipellis reminiscent of a ‘pseudovolva’; colour pure white, whitish to beige or pale straw to pale ochraceous, more rarely lemon yellow (KR-M-0043301, Mu 10YR 8/6), never darkening; surface evenly and densely pruinose all over. Cortina not seen, even in young primordia. Context firm, fibrose, whitish in pileus, whitish or beige to very pale straw in stipe, whitish in the bulb; smell faint, acidulous or more or less pungent, especially when cut; taste not recorded. Colour of excissile: pileus generally uniformly brownish (Mu 10YR 4/3–4/6 or 6/6–6/8), also reddish brown (Mu 7.5YR 4/4–4/6); stipe concolorous or somewhat lighter than pileus (Mu 10YR 5/6–5/8), never showing any distinct darkening (either browning or blackening) upon drying.

Basidiospores (6.7–)7.1–9.8–(10.5) × (5.1–)5.2–7.9–(8.1) µm, Q = (1–)1.1–1.4–(1.5), Lm = 8.2–9, Wm = 6.1–6.7, Qm = 1.3–1.4 [n = 100, from 4 collections], mostly isodiametric to subisodiametric, more rarely subheterodiametric, with nodulose profile which is often ‘marginiform’ or ‘pseudosteniform’ (star-like arrangement), usually presenting quite distinct (7–)9–13–(16), obtuse to subconical knobs, (0.75–)1–1.5–(2) µm high, (1.2–)1.4–1.7–(2) µm broad at the base; apicula distinct, often curved; yellow in ammonium. Basidia 20–32 × 6–11.5 µm, clamp, (2–)4–spored, with sterigmata 3–5–(7) µm long. Pleurocystidia abundant, metuloid, (38–)40–66(–70) × (12–)14–18–25–(26) µm, Q = (1.8–)1.9–2.6–3.4–(3.5) [n = 40], variable in shape among collections, mostly sublageniform, subfusciform to subutiform, often ventricose and extending upwards into a defined neck (see Marchetti et al. 2014: 151).

Fig. 3 Inocybe mixtilis KR-M-0043296: microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = chelocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.
base rounded, not pedicellate or with short rounded pedicel; content hyaline or rarely with orange-yellowish, or yellow greenish amorphous matter; mostly crystaliferous at apex; walls (1–)1.5 µm thick (at the bulge), ~3.5 (~5) µm thick (at the apex), sometimes coalescing along the neck, yellowish in ammonia. Lamellar edge homogeneous and sterile, composed of abundant cheilocystidia and numerous clavate to subovoid paracystidia. Cheilocystidia abundant, similar in shape and size to pleurocystidia (35–)38–50–65 (~67) × (12–)13–17–21 (~26) µm [n = 40], with similar thick walls and crystaliferous apex. Hymenophoral trama regular to subregular, consisting of parallel to interwoven hyphae, 4–15 (~20) µm wide, cylindrical to subfusiform in shape, often constricted at the septa, hyaline. Subhymenium poorly developed, consisting of 2–3 layers of small, subsidiomeric, irregular cells, 4–6 µm diam. Pileipellis constituted by an ixocutis, made up of parallel hyphae, 3–5 µm wide, with finely encrusting and parietal golden to orange yellowish pigment; subcutis with wider and paler elements, up to 20 µm broad; epicutis in young fruitbodies sometimes covered by thin hyaline hyphae, with scattered free ends (belonging to velipellis remains). Stipitpellis consisting of a cutis bearing numerous bundles of caulocystidia similar to hymenial cystidia, sometimes with tapering neck, (35–)42–49–64 (~65) × (13–)14–19–27 (~33) µm [n = 40], mostly sublageniform, subfusiform or subutriform, rarely narrowly clavate or nearly cylindrical, often crystaliferous at apex, intermixed with extremely abundant globose, ovoid to subclavate paracystidia; both elements forming the dense and conspicuous pruina that extends to the bulbous stipe base. Clamp-connections present in all tissues.

Fig. 4 Inocybe mixtilis. a. M-0219661 (epitype designated by Marchetti et al. 2014); b. KR-M-0043296, basidiomata in situ; c–h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from KR-M-0043296). — Scale bars: a–b = 1 cm; c–i = 10 µm; j = 2 µm.
**Habitat & Distribution — Inocybe mixtilis** has a broad eco-
logical range, but shows a marked preference for acid soils, 
sometimes with a sandy or gravelly texture, being also other-
wise frequent in boggy or peaty soils in forests, where the 
epiphyte was encountered. It can be often found in nitified locations, 
such as parks, roadsides, cemeteries or gardens in urban areas 
and also conifer reforestations (e.g., in northern France under 
Pseudotsuga menziesii, GenBank HQ886870, leg. & det. P.A. 
Moreau). Most collections are associated with pure stands of 
coniferous trees, especially in *Picea abies* forests, but also with 
*Pinus* (*P. mugo, P. pinea, P. sylvestris*). One collection from 
Germany grew under *Quercus robur*, and several included in 
the UNITE database (Köjalg et al. 2013) are associated with 
broadleaf trees. All information available suggests that *I. mixtilis* shows a broad host range.

Although certainly mistaken for *I. occulta* and *I. ceskeae, I. mixtilis* is widespread in Europe, but much less common in 
southern Europe. For instance, after extensive sampling in the Iberian Peninsula, only a specimen could be collected in an area 
of Eurosiberian affinity. Interestingly, the southernmost collection 
of *I. mixtilis* was made in La Palma (Canary Islands, Spain) 
under *Pinus canariensis* in laurisilva forest (Macaronesian 
biogeographical Region). Unlike *I. occulta, I. mixtilis* is unknown 
above the timberline in alpine areas, although its presence 
there is probable.

Up to date, *I. mixtilis* is unknown from North America. Current 
information indicates that deposited sequences from North 
American records of *I. mixtilis* correspond to *I. ceskeae* and *I. 
oculta*, in spite that the name *I. mixtilis* appears commonly 
cited in the United States (Smith 1938, Grund & Stuntz 1980, 
1983, Cripps 1997, Matheny 2005, Cripps & Horak 2008). Not 
recorded by Kobayashi (2002) from Asia, a sequence obtained 
from an ectomycorrhizal root, nearly identical to our sequences, 
is present in Iran (UNITE UDB005301).

Additional specimens examined. **AUSTRIA, Oberösterreich, Bezirk Braunau am Inn, Kobernausser Wald, near Schalchen, alt. 525 m, in acid boggy soil with *Sphagnum in mixed forest with *Picea abies, Abies alba and Larix decidua*, 17 Aug. 2014, D. Bandini, J. Christian & L. Quecke (KR-M-0043301); Salzburg, Bezirk Tamsweg, Tamsweg, near Prebersee, alt. c. 1500 m, in boggy acid soil with *Picea abies and Alnus incana*, 14 Aug. 2013, H. Prelicz & B. Halabi (KR-M-0043307). – FINLAND, Etelä-Häme, in park near *Quercus robur*, *Tilia* and *Pinus sylvestris*, 13 Aug. 2008, J. Vauras (TUR-A 182874; duplicate KR-M-0046560); Somero, Hänttälä, W. of the road to Kerkola, NW of Haapasaari farm house, former field planted with *I. mixtilis*, with scattered young trees of *Picea abies, Betula, Salix*, partly mossy and grassy, with abundant litter of *Pinus*, 11 Oct. 1995, J. Vauras (TUR-A 145111; duplicate KR-M-0046559); Varsinais-Suomi, Salo, Helinsunniemi, cemetery, S part, on lawn near *Picea abies*, 16 Sept. 2014, J. Vauras (TUR-A 199995; duplicate KR-M-0046561). – GERMANY, Baden-Württemberg, Alb-Donaun, Illnrieden, TK 7726, on Wiese an Waldrand (grassy soil in forest), 10 Oct. 1978, M. Enderle, det. J. Stangl (as *I. tabacina*) (M-0022251); Landkreis Calw, Neuenweiler, Avenburg, TK 721717, alt. c. 950 m, under *Picea abies*, 19 July 2012, D. Bandini & B. Oertel (KR-M-0043302); Kreis, Freudenstadt, Baiersbronn, Nature Reserve *Wilder Kranz*, *Picea abies* var. *harrisi*, 19 July 2012, D. Bandini & B. Oertel (KR-M-0043301); Kreis, Freudenstadt, Baiersbronn, TK 670633, alt. c. 360 m, boggy soil with *Sphagnum* and *Picea abies*, 2 Oct. 2011, D. Bandini & B. Oertel (KR-M-0043305); Kreis, Freudenstadt, Baiersbronn, TK 670633, alt. at 5 km from the former location, alt. c. 400 m, with *Picea abies*, 6 Oct. 2011, D. Bandini & B. Oertel (KR-M-0043305); Sachsen-Anhalt, Landkreis Harz, Nature Reserve ’Kramerhau’, TK 4229/4, alt. 600 m, boggy soil with *Sphagnum* and *Picea abies*, 1 Oct. 2012, D. Bandini & B. Oertel (KR-M-0043295); ibidem, alt. 680 m, boggy soil under *Picea abies*, 2 Oct. 2012, D. Bandini & B. Oertel (KR-M-0043296); Schleswig-Holstein, Lübeck, TK 2130, Friedhof bei Wein Erle (cemetery with willows and alders), 19 Sept. 1983, H. Glowinski, det. J. Stangl (as *I. tabacina*) (M-0022249). – ITALY, Piemonte, Novara, Fara Novarese, alt. c. 210 m, in clay soil under *Pinus strobus, 9 Nov. 2000, E. Ferrari (EF45/2000, paratype of *I. viscosissima var. bulbosovaleata, Ferrari* 2006). – SPAIN, Basque country, Gipuzkoa, Ametzeta, Ondareko langa, alt. 595 m, under planted *Picea abies and Fagus sylvatica* on calcareous ground, 20 Oct. 2016, D. Bandini & B. Oertel (ARAN-Fung 0471); Islas Canarias, La Palma, Breña Baja, Parque Natural de Cumbre Nueva, Montaña de la Venta, alt. c. 1450 m, in *Pinus canariensis* forest, 15 Dec. 2013, J. Christian (KR-M-0043297). – SWITZERLAND, Graubünden, Ramosch, alt. c. 1300 m, in *Picea abies* forest, 14 Sept. 1995, F. Esteve-Raventos, E. Horak & G. Moreno (AH 40432).

Notes — *Inocybe mixtilis* is undoubtedly one of the most 
cited nodulate-spored species in the genus and has always 
been considered as a common well known representative of 
section *Marginatae* Kühner (1933). Sequence data from 
collections deposited as *I. mixtilis* in GenBank and UNITE are 
very numerous and reflect this fact, as well as the extensive 
iconography and descriptions available in literature (see Materi-
als and Methods).

Stangl (1984) neotyped *I. mixtilis* using a collection from the 
Bavarian location where Britzelmayr first collected and de-
scribed it, but this choice is not correct as Britzelmayr’s original plate (sub *A. rimosus*) was available for lectotypification, as Mar-
chetti et al. (2014) proposed. These authors chose as epitope the ‘neotype’ selected by Stangl (M-0219661). ITS sequence data 
obtained from the epitope have been fundamental to start 
unravel this taxon complex and to prove the existence of 
some closely related species like *I. occulta* and *I. ceskeae*.

The description of *I. mixtilis* given here is based on extensive 
material and complements Stangl’s (1984). The holotype and 
farther material show several distinct diagnostic characters:

1. the rather viscid pileus with a greasy or lardy appearance 
(mostly in wet conditions);

2. spores with protruding knobs arranged often in a ‘margariti-
form’ appearance (hence isodiametric to subsidiodiamic); and

3. cystidial length of about 50 µm in average, their shape being 
normally ventricose and tapering upwards into a very often 
clearly differentiated neck (lageniform to sublageniform).

However, non-typical collections of *I. mixtilis* are difficult to 
morphologically distinguish from *I. ceskeae* and *I. occulta*, which 
have more heterodiametric spores. All three species can be 
found in boreal taiga forests of the Northern Hemisphere.

Two examined collections determined by J. Stangl as *I. tabacina* 
from (M) had an identical ITS region compared to the epitope 
of *I. mixtilis*. This fact provides reliable information regarding 
the cromatic variation of the piles in *I. mixtilis* (see description) 
and its final colour in dry specimens (exsiccat). Specimens of 
*I. mixtilis* with a more brownish orange piles are mistaken for 
*I. tabacina*, especially in dry conditions. However, *I. tabacina* 
shows a tobacco brown to buff brown piles, without any orange 
or yellow colour (in fresh condition), and microscopically both 
species show very different cystidia, being generally slender 
and fusiform in *I. tabacina* and ventricose to utriform in *I. mixtilis*. 

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A sequence obtained from a typical specimen of *I. tabacina* fell outside the *I. mixtilis* complex in our ITS analyses (Fig. 2), and also in previous published analyses (Fig. 1; clade B in Esteve-Raventós et al. (2016).

Our analyses (Fig. 1, 2) also confirm that the paratypes of *I. viscosissima* var. *bulbosovelata* (Ferrari 2006) belong to *I. mixtilis*, but a synonymy is not proposed here since the holotype was not studied (see Specimens examined).

*Inocybe ceskae* Bandini, Esteve-Rav. & B. Oertel, sp. nov. — MycoBank MB819790, ITS barcode GenBank KX290789; Fig. 5, 6

**Etymology.** Named after Oluna Ceska who has made numerous mushroom collections and contributed to the knowledge of fungal diversity in Canada (see notes).

**Holotypus.** FINLAND, Koillismaa, Kuusamo, Oulanka National Park, W of the Research Station, N of the river Oulanganjoki, Lilasenvaaran tie 135, next to gravelled parking place and houses, alt. 166 m, ETRS89 N66°22’10.2” E29°18’37.2”, on sandy and mossy soil, near *Pinus sylvestris*, *Betula* sp. and *Picea abies*, 21 Aug. 2015, D. Bandini, J. Vauras & B. Oertel (TUR-A 203414). Isotypes in KR-M-0046013 and AH 46568.

**Diagnosis:** *Inocybe ceskae* is characterised by a yellowish beige, straw-coloured to pale ochre, smooth and sticky pileus, a thin whitish evanescent velipellis in young basidiomata, shortly heterodiamebical nodulose spores with rather protruding knobs, and short cystidia (average ≤ 50 µm long). *Inocybe ceskae* has been found in one locality in boreal forest in Northern Europe (*Pinus, Betula, Picea*) and also occurs in humid and subtemperate coastal regions of the Pacific North (North America).

**Basidiomes** usually gregarious, rarely solitary. **Pileus** 15–30 mm diam; shape campanulate or obtusely conical to hemi-

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**Fig. 5** *Inocybe ceskae* TUR-A 203414 (holotype), a–c. Basidiomata in situ and in dry condition; d–i. hymenial cystidia; j. spores under optical microscope; k. spores under SEM. — Scale bars: a–c = 1 cm; d–j = 10 µm; k = 2 µm.
spherical, then convex applanate when expanded, sometimes undulate when young, often showing a low and broad umbo, sometimes not or hardly umbonate; margin straight or deflexed, somewhat undulate in expanded specimens, not striate, not distinctly hygrophanous; colour beige-ochre, straw-yellow to pale ochre (Mu 10YR 8/2–8/6, 2.5Y 8/2–8/4), sometimes more intense in colour or with faint orange hue towards the centre, paler and whitish at the extreme margin because of the velipellis; surface radially appressed-fibrillose, smooth and glabrous when young, sometimes becoming finely felty fibrillosate when old, lubricous and sticky in wet conditions, adhering soil and humus particles; velipellis present, distinct in young fruitbodies, as a very thin whitish coating especially at margin but also over the entire pileus, soon disappearing and nearly absent in washed or aged specimens. Lamellae ventricose to subventricose, 2–5 mm deep, nearly free to adnate or adnexed with decurrent tooth, moderately crowded to rather crowded, L = 40–60, l = 1–3; colour initially whitish, beige or greyish, showing a rather distinctive pinkish or purplish hue, especially towards the lamellar edge, then becoming brownish grey or even deep brown to orange or rusty brown when old; edge crenulate, whitish when young, sometimes concolorous with age. Stipe 20–45 × 2–5 mm, solid, fibrose; shape cylindrical to slightly tapering upwards, base most often abruptly marginately bulbous; bulb 6–9 mm broad, agglutinating abundant soil and humus material and sometimes hidden and difficult to perceive; colour whitish when young, then becoming yellowish with age (Mu 2.5Y 8/1–8/4) but not especially browning or darkening; surface evenly and densely pruinose all over, also intermixed with abundant hyaline subclavate paracystidia, (2–)4-spored, with incuriting and intracellular yellowish pigment; hyphae with scattered free ends, from remnants of velipellis. Context whitish to yellowish content rather crystalliferous at apex and sometimes showing a pale yellowish content. Hymenoperiphora trama subregular, constituted by more or less interwoven hyphae, 4–26 µm wide, subcylin- drical, subfusciform to almost ovoid in shape, mostly constriicted at septa, hyaline. Subhymenium poorly developed, consisting of 2–3 layers of small subisodiametrical or irregular cells, 4–6.5 µm diam, hyaline. Pluteipellis consisting of an epicutis, composed of slightly gelatinized parallel hyphae, 4–12 µm wide, with incurtering and intracellular yellowish pigment; hyphae somewhat paler to almost hyaline in deeper layers (subcutis), up to 25 µm wide; epicutis sometimes covered by thin hyaline hyphae with scattered free ends, from remnants of velipellis. Stipitipellis consisting of a cutis of subcylindrical hyphae, constricted at septa, bearing numerous bundles of caulozystidia similar to hymenial cystidia, (29.5–)37.5–47.2–63.5(–67.5) × (17.5–)18.5–21.9–26.5 µm [n = 40], crystalloaphorous at apex, intermixed with abundant hyaline subclavate paracystidia, similar to those of lamellar edge; these elements constitute the dense and abundant pruina reaching the bulb. Clamps connections present in all tissues.

Habitat & Distribution — The holotype collection was found in Finland on sandy soil, next to a gravelled parking place in an anthropogenic area, but it is unknown to us whether the soil had calcareous profile. Several Canadian collections (British Columbia) were also gathered in human-influenced locations (see Notes), while our two European collections (holotype and paratype) were made in taiga forest, suggesting that I. ceskae is associated with conifers (Pinus or Picea) with the presence of Betula species. In the Pacific Coast of Canada (British Columbia) and north-western USA (Oregon), I. ceskae is associated with Pseudotsuga menziesii (Douglas-fir) and probably also with Tsuga heterophylla (western hemlock). Inocybe ceskae occurs further south along the Western Cordillera, and reaches Sierra Nevada Foothills (Northern California), where it is probably associated with Arbutus menziesii and Quercus. (Smith et al. 2002, Morris et al. 2008). Kennedy et al. (2012) have assessed the potential for the existence of common mycelial networks between A. menziesii and P. menziesii; Arbutus menziesii hosts a highly diverse mycorrhizal fungal community with similar composition to those found on other angiosperm and Pinaceae hosts (including P. menziesii). This fact could explain why I. ceskae has been found forming ectomycorrhiza with A. menziesii and some oaks in more southern and subtemperate areas of Northern California (Morris et al. 2008).

Due to its transoceanic distribution, it is very probable that I. ceskae extends throughout boreal to subboreal taiga forests in both continents and also Siberia.

Additional specimen examined. FINLAND, Kolllismaa, Kuusamo, Oulanka National Park, west of the Research Station, N of the river Oulanganjoki, Liikasenvaarantie 135, next to gravelled parking place and houses, alt. 166 m, ETRS89 N66°22′10.2″ E29°18′37.2″, on sandy and mossy soil, near Pinus sylvestris, Betula sp. and Picea abies, 26 Aug. 2016, J. Vauras (paratype KR-M-0042321; isoparatype TUR-A 203863 (Vauras 213686F))).

Fig. 6 Inocybe ceskae TUR-A 203414 (holotype): microscopic features. Sp = spores, Pa = parazystidia, Cpa = cauloapatridia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.
Notes — Our description is based on the holotype and the paratype, both gathered in the same spot in consecutive years. These materials are conspecific with eleven collections at UBC and all form a strongly supported monophyletic clade (Fig. 1). The UBC materials were mostly determined as *I. mixtilis* from Canada (see Table 1) by Oluna Ceska, after whom we name this species. Photographs, drawings and notes of these Canadian vouchers are available on the internet (www.biodiversity.ubc.ca/museum/herbarium/). All of them were gathered in different localities in British Columbia, alt. 30–240 m, in the so-called Marine West Coast Forests, characterized by a temperate and moist climate. In all UBC material in which the ecology is noted the ectomycorrhizal host is *Pseudotsuga menziesii*, except for two specimens collected under *Tsuga heterophylla* (F19812) and *Arbutus menziesii* (F19202), respectively.

Seven of these collections at UBC were identified as *I. mixtilis* and actually belong to *I. ceskae*. The other four were originally determined as *I. glabrodiscia*, *I. praetervisa*, *I. sindonia* and *I. umbilica*. These identifications suggest that the pileus colour can be pale and interestingly, the fruitbody named *I. glabrodiscia* (F19553) is, as explicitly told, a very aged one, and apparently brownish.

Unfortunately, the examination of these UBC materials was not authorized while undertaking this study and this has refrained us from designating a North American collection as the holotype.

According to Oluna Ceska’s drawings and annotations, there must be a certain range of variation in macro- and micromorphological features, but the resemblance with the holotype is obvious:

1. the range of pileus size in the Canadian vouchers is 25 (F23607) to 55 mm diam (F18984);
2. most exsiccata show a light yellowish to pale straw-coloured pileus, mostly broadly umbonate, and the surface is often shiny and glabrous, with adhered soil particles suggesting that they were lubricous or viscid;
3. fresh pileus colour indicated by Oluna Ceska ranges from ochre (F18954) to yellow-ochre or yellow-brown, or darker yellowish brown or even very pale (F18984). The centre of the pileus in one collection is described as ‘with an ochre-orangy patch’ (F18974);
4. an obtusely umbonate pileus is present in photos and sketches of most fruitbodies;
5. lamellar colour is whitish brown to greyish brown turning to dark olive (F18974);
6. the colour of the stipe is white, ‘white silky’ or whitish with a yellow tinge (F18984);
7. the stipe is always depicted pruinose over the entire length, and a marginate bulbous base is distinct in F19221 (photograph of fresh specimens) and also in Ceska’s sketches (e.g., F18984); and
8. the stipe length is variable among these collections, up to 80 mm long (F18954) and –8 mm wide, with a bulb up to 11 mm (F18984). On the contrary, basidiomata in F23607 are very slender, with pileus up to 30 mm diam, with a prominent umbo and stipe up to 40 × 2.5–4 mm.

Unfortunately, microscopic features are roughly drawn. Spores show distinct, often prominent knobs, with measurements 8–10–11 × 6–7 µm (F18954), 8–9–10 × 6–7 µm (F18974), 7–9–5–6.5 × 6–7–8–9 µm (F18984) and 8–11 × 6–7 µm (F19553). Cystidia are ventricose with an attenuated obtuse neck and thick walls (–3 µm), but their base is never depicted. Measurements are only indicated for three collections: 45–55 × 12–13 µm (F18954), 45–50 × (10–13–20 µm (F19553) and 45–50 × 15–22 µm (F19553), which reflects the small size and variable width of cystidia.

Although somewhat paler in colour, the habit of *I. ceskae* is similar to *I. mixtilis* and *I. occulta*. *Inocybe mixtilis* is shown to be sister to *I. ceskae* in our ITS-RPB2 phylogeny (Fig. 1), but it differs from the latter in showing a more intensely yellow to orange pileus, lubricous or sticky in wet conditions, and, microscopically, a large proportion of the spores have protruding obtuse knobs that give the spores a ‘pseudoasteriform’ or ‘margaritiform’ shape. Some collections of *I. ceskae* and *I. occulta* may share a thin whitish velipellis, which is mostly ephemeral and observed in young specimens not washed by rain, especially at pileus margin. Nevertheless, both species are sometimes difficult to recognize morphologically, but *I. ceskae* shows paler colours and shorter cystidia in average. *Inocybe ceskae* and *I. occulta* can occur in similar habitats, being *I. occulta* more widespread geographically, also extending to warmer and more temperate climates in Europe and North America. *Inocybe johannis-stanglii* and *I. nothomixtilis* show distinct heterodiametrical spores with a lower ornamentation. Furthermore, *I. johannis-stanglii* has a greyish velipellis and larger spores and cystidia, while *I. nothomixtilis* prefers instead southern, subtropical to warm ecosystems where it is associated mainly with *Fagaceae* and *Cistaceae*.

Based on material from Kamchatka (Eastern Siberia, Russia), Peintner & Horak (2002) attributed to *I. mixtilis* a pale-coloured pileus, heterodiametric spores provided with distinct knobs and short cystidia, that suggest *I. ceskae* instead. This fact substantiates our hypothesis of *I. ceskae* being more widespread over circumpolar regions of the Northern Hemisphere.

**Inocybe johannis-stanglii** Bandini, Esteve-Rav. & G. Moreno, sp. nov. — MycoBank MB819791. ITS barcode GenBank: KX290791; Fig. 7, 8

**Etymology.** Named after Johann Stangl, for his great contribution to the knowledge of the genus *Inocybe*.

**Holotypus.** GERMANY, Baden-Württemberg, Heidelberg, TK 65183, alt. 120 m, churchyard of Johanneskirche, on lawn with *Picea omorika* and *Betula pendula*, 4 July 2012, D. Bandini, KR-M-0038039. Isotype AH 6580.

**Misapplied names** — *Inocybe pallida* Velen. sensu Stangl, Hoppea 46: 308. 1989.

Selected descriptions and iconography: Stangl (1989: 308, Taf. 34/2); Ferrari et al. (2014: 106, 175)

**Diagnosis:** *Inocybe johannis-stanglii* is characterized by: a) the distinctly heterodiametric spores, measuring (7.6–)8.4–10.1–12–13 × 6–7–8–9(–8.2) µm, Q = (1.2–)1.3–1.5–1.8–1.9 µm, provided with numerous (8–16), low and rounded knobs; b) thin greyish velar patches on the pileus; c) abruptly bulbous stipe, not always distinctly marginate; d) intense lilac hues on lamellae when young; and e) cystidia often tapering into a distinct pedicel. Associated with conifers in central Europe in natural to semi-natural woodlands areas.

**Basidiomes** usually gregarious, rarely solitary. *Pileus* 10–30 mm diam; shape when young campanulate, subconical to hemispherical, then convex to expanded when old, sometimes somewhat undulate or irregular, sometimes with a broad low obtuse umbo; margin mostly straight, occasionally deflexed; colour when young pale ochre, then mostly dull brown (*Mu* 10YR 6/6–7/8), brownish ochre or hazel brown (*Mu* 10YR 6/6–6/8 or 5/6–5/8), or, due to the presence of velipellis greyish brownish, entirely or only at margin and umbo; surface glabrous or finely felted to finely fibrillose with adpressed, not diverging fibres, in some collections slightly hygrophanous (e.g., KR-M-0043321); often lubricious in wet conditions and with adhering soil particles; velipellis distinct, especially in young fruitbodies, as a thin greyish layer covering the pileus centre or as scattered patches.
Lamellae ventricose to subventricose, 2–5 mm deep, almost free, adnexed, emarginate-adnexed or emarginate with decurrent tooth, moderately crowded to crowded, \( L = 40–60, I = 1–2 \); colour initially whitish, very often with a pale vinaceous grey, greyish lavender or light lilac tinge, finally greyish brownish, sometimes with a lilac tinge; edge fimbriate, whitish to even concolorous in old basidiomata. Stipe 20–40 × 2–4 mm, solid, fibrose; shape cylindrical or slightly tapering upwards, base bulbous or often faintly marginately bulbous; bulb up to 8 mm broad, mostly rounded marginate, in some collections hardly or not marginate and apparently clavate bulbous (e.g., in KR-M-0043320); colour whitish when young, then also wax-coloured or very pale brown at most (Mu 10YR 8/3–8/4), never showing a strong darkening when bruised or with age; surface evenly and densely pruinose all over, reaching the bulbous base.

**Colour of exsiccata:** pileus rather uniform light brown, greyish brown (Mu 10YR 5/4–5/6, 10YR 4/4), stipe beige to straw yellow (10YR 7/4–7/6) or yellowish brown (Mu 10YR 6/4), never showing any distinct darkening upon drying. Cortina not seen, even in young specimens. Context fibrose, firm, whitish in the pileus and also in the stipe when young, then with a brownish tinge, especially in the upper half and the cortex of the stipe; smell indistinct, weakly spermatic when cut.

**Basidiospores** (7.6–)8.4–12(–13) × 6–7.8(–8.2) µm, \( Q = (1.2–)1.3–1.8(–1.9) \), \( L_m = 8.3–9.1, W_m = 6.6–7.1, Q_m = 1.4–1.5 \) [n = 100, from 4 collections], heterodiametric, with nodulose profile, presenting (8–)10–16 low, rounded to broadly conical knobs, 0.4–1.2(–1.4) µm high, 1–2(–2.2) µm broad at base; yellow in ammonia, without germ pore and sometimes with a large central guttule. **Basidia** 22.5–32 × 8.5–12 µm, clavate,
4-spored, in some collections mixed with 2-spored, with sterigmata 2.5–6 µm long. *Pleurocystidia* abundant, metuloid, (35–)45–55–63(–68) x (11–)13–17.5–21–24 µm, Q = 1.9–2.5 [n = 40], subutriform, subagenomen, also sub fusiform, occasionally subcylindrical or subclavate, often rather ventricose, mostly with rather short obtuse neck, base often tapering into a more or less distinct, sometimes curved pedicel; apex usually rather crystalliferous; walls 1–2.5 µm, often thickened towards the neck, up to 3.5 µm thick, yellowish in ammonia; content generally hyaline, but sometimes showing a pale yellow to glaucous amorphous content in ammonia solution. *Lamellar edge* almost homogenous and sterile, consisting of numerous hyaline, mostly hyaline, but sometimes showing a pale yellow to glaucous amorphous content in ammonia solution. The neck, up to 3.5 µm thick, yellowish in ammonia; content generally hyaline, but sometimes showing a pale yellow to glaucous amorphous content in ammonia solution.

***Additional specimens examined. AUSTRIA***, Oberösterreich, Bezirk Braunau am Inn, Kobermauser Wald, near Schalchen, alt. c. 525 m, mixed forest with Picea abies, Abies alba, Larix decidua, 17 Aug., 2014, D. Bandini, J. Christian & L. Quecke (KR-M-0043320); in about 2 km distance from the former location, alt. c., 550 m, mixed forest with Picea abies, Abies alba, Larix decidua, 17 Aug. 2014, D. Bandini, J. Christian & L. Quecke (KR-M-0043321). **GERMANY**, Bayern, Augsburg-Bergheim, TK 7631, garden of A. Sedelmeier, with Beltra and Picea omonika, 28 Oct. 1987, J. Stangl (M-0021484, as *I. pallida sensu Kuyper*); 9 Nov. 1987, J. Stangl (M-0021483, as *I. pallida sensu Kuyper*); Baden-Württemberg, Heidelberg, TK 6518/3, alt. 120 m, churchyard of Johanneskirche, on lawn with Picea omonika and Beltra pendula, 18 July 2012, D. Bandini (KR-M-0046122); 19 July 2012, M. Rave (KR-M-0038082); 31 July 2014, D. Bandini (KR-M-0042290, duplicate in TUR-A 203094).

**Notes — *Inocybe johannis-stanglii*** is a well-delimited species within the Mixtilis group, as revealed by our analyses (Fig. 1, 2). Morphologically, the presence of a rather distinct velipellis and the hyaline cystidia of this species is one of the largest known in this group, (often longer than 10 µm), are diagnostic. Spores of *I. johannis-stanglii* recall those of *I. nothomixtilis* and *I. subtrivialis* in shape and ornamentation, with low but distinct numerous knobs. The hymenial cystidia, in average longer than 50 µm, are also very particular, because of having a base tapered into a pedicel. Caulocystidia are also abundant near the bulb of the stipe, which is not distinctly marginate in some specimens.

The presence of a distinct velipellis has also been depicted by Stangl (1989, Taf. 34/2) from two collections deposited at M that were seen by T.W. Kuyper and determined as *I. pallida* by the latter. Both collections have identical ITS sequences compared to the holotype of *I. johannis-stanglii*. Stangl (1989) named both collections as *I. pallida* following Kuyper (1985), who revised Velenovsky’s *Inocybe* collections at PR and PRC. Velenovsky (1920) originally described and depicted *I. pallida* with a narrow stipe (1–2 mm), progressively enlarged towards the base, but never really bulbous, and angulose spores in outline. The material was gathered in a xerophilous open grassland habitat, with presence of Helianthemum, Thymus, Phleum and Trifolium. This particular habitat suggests a possible relationship with the ectomycorrhiza-forming *Helianthemum* (Cistaceae), common in heliophilous and warm habitats in Mediterranean and continental European areas. Some years later, Velenovsky (1947) gave a Latin diagnosis of *I. pallida* and added some microscopical data, like spores ‘12–14 µm, cuneatae, angulato-aculeatae’, cystidia measuring 50–60 × 25–40 µm, and habitat ‘in gramineum, in fruiticibus Ruborum non raro’. Kuyper’s revision (1985: 394 and f. 33) of the holotype of *I. pallida* confirmed the angulose outline of the spores (‘with indistinct nodulae’), the very wide cystidia × 20–24(–26) µm, with walls up to 1.5 µm thick and caulocystidia descending over halfway of stipe. Kuyper also suggested that *I. pallida* is related to *I. decipiens*, but differed in the ‘smaller, more angular spores’.

The original concept of *I. pallida* remains difficult to interpret with certainty and sequencing of type material is necessary to infer its position among the nodulose/angiulate-spored *Inocybe* species. Nevertheless, this is probably impossible to achieve, since the holotype is preserved in a liquid solution (Kuyper 1985), which is a mixture of formaldehyde, acetic acid and water (Antonín 1986, 1990), and its DNA is presumably highly degraded. Thus, an epitype for *I. pallida* should be selected, preferably using material from the type locality, in order to establish a consistent interpretation.

**Fig. 8** *Inocybe johannis-stanglii* KR-M-0038039 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pi = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.
In the meantime, morphological and ecological features of the holotype of *I. pallida* strongly suggest that this taxon could be a member of the *I. decipiens/dunensis* complex due to the angular spores (with hardly protruding knobs, if any), large ventricose utriform cystidia without tapering base, an indistinctly bulbous stipe, caulocystidia only descending halfway of the stipe and a xerophilous habitat. All of this suggests a closer relationship with the *I. decipiens/dunensis* complex, as earlier suggested by Esteve-Raventós & Caballero Moreno (2009: 112). Sequences identified as *I. pallida* in GenBank show that this name has been consistently employed for species of *Inocybe* of the *I. decipiens/dunensis* group.

**Inocybe nothomixtilis** Esteve-Rav., Bandini & V. González, *sp. nov.* — MycoBank MB819792; ITS barcode GenBank KX290780; Fig. 9, 10

*Etymology.* From Latin *nothus*, which means false, and *mixtilis* (*Inocybe*), which represents a different but very close species.

*Holotypus.* Spain, Basque Country, Araba (Alava), Zuia, Markina, in acid, peaty soil, among *Sphagnum* spp. and other mosses near *Erica tetralix* in a *Quercus robur* stand, with some reforested pines (*Pinus radiata*) and *Sorbus aria* nearby, 29 Aug. 1996, R. Fernández-Sasia, RFS no. 960829-01 (AH 24510). Isotypes KR-M-0046551 and in CEME-EMI (Centro de Estudios Micológicos de Euskadi-Euskadiko Mikologia Ikastegia).

≡ *Inocybe tabacina* var. *pseudovolvata* Eyssart. *ad int.*, Bull. Soc. Mycol. France 122: 177. 2006 [not validly published].

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**Fig. 9** Inocybe nothomixtilis. a. AH 24510 (holotype) *in situ*; b. KR-M-0043322 *in situ*; c–h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from AH 24510, holotype). — Scale bars: a–b = 1 cm; c–i = 10 μm; j = 2 μm.
Diagnosis: Macroscopically I. nothomixtilis resembles I. mixtilis in habit, size and colours, but it is distinguished by: a) a developed whitish velpellis, easily observed on the pileus and the edge of the bulb in primordia or young specimens; b) the heterodiametric basidiospores 7–8.5–10.1(–11) × 5–5.9–6.8(–6.9) μm, Q = 1.2–1.45–1.7(–1.8) [n = 100, from 4 collections], with 10–13 low, obtuse and well-delimited knobs; c) rather short cystidia of average length ≤ 50 μm; d) pileipellis not viscid or slimy, but sticky to lubricous in wet conditions, or even dry. Distributed in temperate continental and Mediterranean regions of Europe.

Basidiomes usually gregarious, rarely solitary. Pileus 15–30(–35) mm diam, 5–17 mm high, when young broadly conical to hemispherical campanulate, then expanded to broadly convex, convex or more rarely aplanate, often with a broad and low umbo; margin slightly inflexed when young, then more straight upon expansion, initially entire or regular, then somewhat wavy to irregular in old specimens, not striate, not or very slightly hygrophanous in old or very soaked fruitbodies; colour somewhat variable, but always showing a range of yellow colours, from golden yellow to brownish yellow or orange-yellow in young specimens (Mu 10YR 8/6; 7.5YR 7/6–7/8), often brownish to reddish yellow or brownish with a reddish orange hue in older specimens (Mu 7.5YR 6/8), sometimes pale yellowish to ochraceous, especially towards the margin (Mu 10YR 8/6 or 7/8); surface radially fibrillose, most often feltly to even woolly-fibrillose with bunches of adpressed fibres, sometimes becoming glabrous towards the centre or in some collections even practically smooth in its entire surface (e.g., in I. tabacina var. pseudovolvata), lubricious and sticky but not viscid in wet conditions, with adhered soil and humus particles; velpellis clearly visible in young fruitbodies as a thin to somewhat thick whitish coating on a yellowish background, rather persistent, sometimes lanose-fibrillose and still visible at centre and/or margin in old specimens. Lamelae ventricose to subventricose, 2–4(–5) mm deep, nearly free or adnexed, moderately crowded, L = 40–48(–50), I = 1–2(–3); colour initially white or whitish, soon becoming beige or pale grey, brownish grey and finally ochre-brown, in some collections (e.g., in the holotype) up to 3–4 mm at the neck and the extreme apex, and then sometimes walls coalescing (like a ‘plug’), hyaline to very pale yellow in ammonia; content hyaline, never showing dark (brown to dark brown) pigments, but sometimes filled with a hyaline to pale yellowish amorphous content. Lamelae edge homogeneous and sterile, composed of numerous to scattered cheilocystidia and very abundant clavate to ovoid paracystidia, mixed with intermediate elements in shape, hyaline, occasionally pale yellow mostly in aged specimens. Cheilocystidia similar in shape and size to pleurocystidia, (26–)30–44–57(–60) × (10–)12–16–21(–24) μm [n = 40], with similar thick walls and crystalliferous apex. Hymenophoral trama regular to subregular, constituted of more or less parallel hyphae, 4–20(–25) μm wide, cylindrical to fusiform in shape, often constricted at the septa, hyaline or with pale yellow intracellular pigment. Subhymenium poorly developed, consisting of 1(–2) layers of small, subdiscomatous cells, 4–6 μm diam. Pileipellis consisting of an epicutis, somewhat gelatinized (but not a distinct ixocutis), constituted by parallel hyphae up to 4 μm wide, with finely encrusting and intracellular parietal yellowish pigment; hyphae somewhat wider and paler in the subcutis, up to 20(–25) μm wide; the epicutis is sometimes covered by thin hyaline hyphae, with scattered free ends (velpellis remains). Stipitipellis consisting of a cutis bearing numerous bundles of caulocystidia similar to hymenial cystidia, (26–)30–41.5–53(–60) × (12–)13–15.5–19(–20) μm [n = 40], crystalliferous at apex, and intermixed with numerous paracystidia, similar to those of the lamellar edge (both elements forming a dense and conspicuous pruina), and clearly reaching the bulbous stipe base. Clamp connections present in all tissues.

Habitat & Distribution — In humus of warm or temperate natural forests of Fagaceae (Fagus sylvatica, Quercus spp.) and Cistaceae stands (i.e., Cistus monspeliensis, C. salviifolius), sometimes mixed with Pinus spp. Inocybe nothomixtilis is also

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**Fig. 10** Inocybe nothomixtilis AH 24510 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.
found in parks or urban areas and then in mixed forests with broad-leaved trees (e.g., *Betula, Carpinus, Tilia*), or conifers (e.g., *Pinus, Abies*). It prefers acid to calcareous soils with sandy texture. Its current known distribution includes France, Germany, Italy and Spain, but considering its habitat preferences, it is probably more widespread in Mediterranean and sub-Mediterranean European areas, and also in warm and temperate locations in continental Europe.

Additional specimens examined. FRANCE, Ile-de-France, Seine-Saint-Denis, Parc du Sausset, under mixed broad-leaved trees (Fagus sylvatica, Carpinus betulus, Quercus spp.), 25 Oct. 2006, G. Eyssartier, GE 06-110 (PC 0738072, as *I. tabacina var. pseudovolvata*). – GERMANY, Baden-Württemberg, Karlsruhe, TK 691/3, alt. 118 m, in a lawn with *Quercus petrea*, *Fagus sylvatica* and *Betula pendula*, 1 June 2013, D. Bandini & B. Oertel (KR-M-0038133); Hessen, Landkreis Bergstraße, Bürsstadt, Waldfriedhof Lampertheim, TK 6317/3, alt. 95 m, in acid sandy soil under *Pinus sylvestris* and *Quercus rubra*, 26 Oct. 2014, D. Bandini (KR-M-0043131, duplicate in TUR-A-203452); – ITALY, Toscana, Castiglion Fiorentino, St. Stefano, alt approx. 500 m, in Mediterranean forest near Quercus spp. (possibly Q. pubescens or Q. cerris), in presumably decalcified soils, 18 Oct. 2010, M. Dondli (KR-M-0043322). Locality unknown (probably Piemonte, Pamparato according to Alessio & Rebaudengo (1980), under Abies alba (?) 24 June 1986, C.L. Alessio, (private herbarium of C.L. Alessio, as *I. mixtilis var. aurata*), – Spain, Cataluña, Barcelona, Collserola, Can Ferrer, in Cistus bushes stand (*C. monspeliensis* and *Pistacia lentiscus*, in decalcified washed soils, 26 April 1994, J.L. Siquier, ILS 7158 (AH 46558)).

Notes — Habit and size of *I. nothomixtilis* fruitbodies are very similar to other taxa in the *Mixtilis* group, but *I. nothomixtilis* is characterized by its well-developed and rather persistent veil, at least in primordia and young specimens, as well as by its regularly felted to rather woolly-fibrillose pileus in most collections. This whitish veil, when not washed out by heavy rainfall, is easily observed on the pileus and on the bulb edge, forming here a 'pseudovolva'. In wet conditions, this velipellis covers a well-developed velipellis forming a 'pseudovolva' around the bulb edge. As mentioned above, this character is very helpful to recognise *I. nothomixtilis*, especially when basidiomata are young and in good condition. Eyssartier also provided some interesting macrochemical reactions not tested by us: KOH 5% yellow on pileus flesh and orange brown on pileus surface; silver nitrate chocolate brown after 10–15 min on pileus flesh. *Inocybe tabacina var. pseudovolvata* was never validly published, but a detailed description was provided. We confirm here by examining the collection kept at PC that it matches *I. nothomixtilis* in most characters, especially in the heterodiametric and small spores (*Lx* = 8.4 × 6; *Qm* = 1.4, *n* = 21) and in having short cystidia (*L* < 50 µm). Eyssartier also pointed out some similarities between its variety and *I. mixtilis* but stated that *I. tabacina var. pseudovolvata* was distinguished by a well-developed veil and a darker pileus colour. However, no comparison of microscopic characters was made by him.

*Inocybe mixtilis var. aurata* was introduced by Alessio & Rebaudengo (1980: 302) to include some collections with a more yellow golden colour and a drier piles ('pili curiculari aridoarem . ...'). Alessio described the pileus as practically dry, hence devoid of the characteristic slimy cuticle which is evident in wet conditions in *I. mixtilis*. This var. aurata was not validly published because two different gatherings were designated as the holotype (Art. 40.1). Moreover, no original material (Pamparato, Cabanet in 1972 and Roburentello in 1976) is located in E. Rebaudengo's private herbarium (Ceva, Italy), where it was said to be deposited.

Although most Alessio's *Inocybe* collections used for his monograph are lost (M. Candusso and E. Bizio, pers. comm.), we were able to study three authentic (but not original) collections of var. aurata kept in Alessio's private herbarium, sent to us by M. Candusso. The material is poorly preserved, mouldy and lacks accurate collection data. Two collections could be ITS–sequenced, one of them belonging to *I. mixtilis* (24 June 1986) and the other one conspecific with *I. mixtilis* (15 Sept. 1984). DNA extraction was successful and the ITS sequence obtained and analysed. The specimen dated 24 June 1986, 'prato nostro sotto abete bianco' is conspecific with *I. nothomixtilis*, while the one dated 15 Sept. 1984, belongs to *I. mixtilis*. A third collection also on 24 June 1986 is a leiosporous species. From these facts, we conclude that Alessio's concept of var. aurata was heterogeneous.

**Inocybe occulta** Esteve-Rav, Bandini, B. Oertel & G. Moreno, sp. nov. — MycoBank MB819793; ITS barcode GenBank KX290787; Fig. 1, 12

Etymology. From Latin *occultus*, which means hidden or concealed, due to the likely confusion with similar taxa of the *Inocybe mixtilis* group.

Holotypus. **SPAIN, La Rioja, Villarroya, 800 m, 30T WM76, on washed and calcareous sandy soil, in a Quercus ilex subsp. balsata forest, 17 May 2008, A. Caballero, AC3805 (AH 36443).** Isotype KR-M-0046550 and A. Caballero's private herbarium.

Selected description and iconography: Esteve-Raventós & Caballero Moreno (2009: 37, f. 11, photos 27–31 – as *I. mixtilis*).

Diagnosis: *Inocybe occulta* is morphologically rather close to *I. ceskae*, differing in the more intense yellow-orangish to orange-brown pileus colour and the slightly longer cystidia in average. *Inocybe occulta* is sister to *I. nothomixtilis* in our ITS-RPB2 analyses (Fig. 1), but it differs primarily from the latter in having more isodiametric spores with lower knobs. *Inocybe ceskae* and *I. mixtilis*, both with spores of similar shape (*Qm* = 1.3–1.4) to *I. occulta*, cluster in a different clade and are phylogenetically distinct from *I. occulta* (Fig. 1).
Basidiomes usually gregarious, rarely solitary. Pileus (12–)15–30(–45) mm diam, shape initially campanulate or obtusely conical to hemispherical, then convex applanate when expanded, often with a low and broad umbo, but occasionally not or hardly umbonate; margin straight to slightly deflexed, sometimes wavy in expanded specimens, not striate, only slightly appendiculate by fugacious velar remnants when present; colour variable, often yellow to orange, dull to more or less bright, e.g., beige, yellowish ochre, buff yellow to golden yellow, orange-yellow to orange-brown (Mu 10YR 8/4–8/8, 7/6–7/8, 7.5YR 7/6–7/8 or 6/6–6/8), in some collections also light brown (Mu 10YR 5/6–5/8), at least towards the margin (e.g., KR-M-0046554 or KR-M-0046555), uniform or very often slightly darker at the centre or the umbo; surface radially appressed-fibrillose, smooth and glabrous, sometimes with a micaceous and/or a falsely hygrophanous appearance (e.g., KR-M-0046556), even not clearly rimose towards the margin, with lubricous aspect in wet conditions, somewhat sticky and hence often with adhering soil and humus particles; velipellis absent or scarcely developed, in this case fugacious but still observable in young specimens of some collections, especially towards the periphery and margin, forming small fibrillose patches or squamules, but mostly absent in many collections when washed by rainfall or aged. Lamellae ventricose to subventricose, 2–6 mm deep, practically free or hardly adnexed, often crowded, sometimes moderately crowded, \( L = 45–55 \), \( l = 1–2 \); colour initially whitish to beige or greyish, often showing a lilac to violet reflection (‘Psathyrella-like’), when old becoming ochraceous to brownish grey and finally yellow-brown to orange-brown; edge finely fimbriate to crenulate, whitish or paler than the sides. Stipe
20–50(–55) × (2–)3–6(–8) mm, solid, fibrose; shape cylindrical to tapering upwards, base distinctly marginately bulbous, often agglutinating soil or humus debris; bulb 7–10(–12) mm broad, edge often clear-cut and even volviform, but velar rests absent or ephemeral in young stages; colour white or dirty white when young, often turning to ochreous, ochreous yellow or even bright yellow in some collections (e.g., AH 36148), or fawn with age (Mu 2.5Y 8/1–8/4), never browning nor blackening; surface evenly and densely pruinose all over, up to the bulb. Cortina not seen, even in young primordia. Context fibrose, firm, whitish, concolorous towards the cortex in pileus and stipe, hardly becoming yellowish with age or when drying; smell generally faint in fresh specimens, often indistinct, in some collections more or less pungent when cut, exceptionally aromatic or even honey-like (AH 36443); taste not recorded. Colour of exsiccata in fresh specimens, often indistinct, in some collections more coming yellowish with age or when drying; smell generally faint concolorous towards the cortex in pileus and stipe, hardly be-seen, even in young primordia.

**Basidiospores** (7.2–)7.5–10(–10.5) × (5.2–)5.3–7.5 µm, Q = (1.1–)1.15–1.57(–1.63), Lₘ = 8–9.6, Wₘ = 5.9–7, Qₛ = 1.3–1.5 [n = 100, from 4 collections], variable in shape (even within a specimen), from heterodiametrical to shortly heterodiamentrical and less frequently subsbiodiametrical; spore ornamentation somewhat variable, mostly presenting 8–13 conical obtuse knobs, (0.9–)1.1–1.4(–1.7) µm high, 1.2–1.6 µm broad at base (but in KR-00043312 many spores show low knobs or even a polyhedral angulate outline, `sub-entolomatoi'); apicula distinct; without germ pore and sometimes with a central oil guttule. **Basidia** 22–28(–30) × 8–10 µm, clavate, 4-spored, with sterigmata 3–5 µm long. **Pleurocyostidium** abundant, metuloid, (37–)39–51.5–64(–67) × (11.2–)11.5–17–22(–24) µm, Q = 2–3–4.1 [n = 40]; of variable shape among collections, normally ventricose fusiform to subtrumiform, occasionally ventricose sublageniform with obtuse apex and short neck, but also not distinctly ventricose in some collections; base normally not pedicelate, tapering into an obtuse base, seldom truncate; rather crystalliferous at apex in most collections; walls (1–)1.5–2.5 µm, often thickened towards the apex up to 3–4 µm, not coalescing at the neck, pale yellow in ammonia; content hyaline or pale yellowish, not showing dark pigments. **Lamellar edge** homogeneus and sterile, composed of numerous cheilocystidia and rather abundant small clavate to subovoid paracystidia, 7–12 µm broad. **Cheilocystidium** abundant, similar in shape and size to pleurocyostidium, 33–47–61–65 × 11.5–17.5–23 µm [n = 40], with similar thick walls, rather crystalliferous at apex, hyaline or showing a pale yellowish content. **Hymenophoral trama** subregular, consisting of more or less parallel to inter-woven hyphae, 3–12 µm wide, subcyllindical to subfusiform, hyaline. **Subhymenium** poorly developed, consisting of 2–3 layers of small branched to subisodiametric or irregular cells, 3.5–5 µm diam, hyaline. **Pileipellis** consisting of an epicutis of slightly gelified pellis hyphae, 3.5–15 µm wide, with incrusted and intracellular yellowish pigment; hyphae somewhat paler to almost hyaline in deeper layers (subcutis), up to 20(–25) µm wide. **Stipitipellis** consisting of a cutis of subcyllindical hyphae, often constricted at septa, bearing numerous bundles of caulo-cystidia similar to hymenial cystidia, (27–)30–48.5–66.5(–69) × (11–)11.5–17–22.5 µm [n = 40], crystalliferous at apex, intermixed with abundant hyaline, ovoid to subclavate paracystidia (x 8.5–18 µm), similar to those of lamellar edge; these elements constitute the dense and abundant pruina covering the whole stipe. **Clamp connections** present in all tissues.

Habitat & Distribution — **Inocybe occulta** occurs in a broad range of habitats and is distributed from cold boreal areas to warm Mediterranean regions of the Northern Hemisphere. Many collections were made in boreal to subboreal taiga forests in Europe (mixed forests including *Picea, Larix, Betula, Pinus*, etc.), but also in warmer and temperate habitats in central and southern Europe in association with conifers (*Picea, Larix, Pinus*) or broad-leaved trees like *Fagaceae* (*Fagus sylvatica, Quercus robur*, etc.). **Inocybe occulta** also occurs in more xeric Mediterranean habitats, in association with *Pinaceaee* (*Pinus pinaster, P. pinea*, etc.). *Fagus* (evergreen oaks like *Quercus ilex, Q. suber*, etc.) and also *Cistaceae* (*Cistus spp.*). Interestingly, *I. occulta* is also present at the alpine stage in the Pyrenees, in *Dryas octopetala* and dwarf Salix communities on slightly calcareous soils. These data suggest that, unlike *I. cesaeae* and *I. mixtilla*, *I. occulta* has probably a more extensive distribution and broader range of hosts. The known distribution of *I. occulta* in North America overlaps that of *I. cesaeae*, being known in subtemperate coastal *Pseudotsuga* forests from the Northern Pacific (British Columbia, Canada) to Oregon (USA), here in young *P. menziesii* forests (Smith et al. 2002). According to sequences available in GenBank, *I. occulta* is found in Mexico (Morelos, Volcán El Pelado), presumably in native *Pinus* forests (*KC152132*) and also in Australia (Canberra), introduced with *Pinus* (KP308781).

**Inocybe occulta** does not show specific soil pH requirements. Many of the collections from montane areas in northern and continental Europe were gathered in calcareous soils, but it also occurs in calcifuge, neutral to slightly acid soils with sandy texture in Mediterranean areas, (e.g., in evergreen-oak or Mediterranean pine forests, like *Pinus pinea* in Italy, ref. GenBank JF908141, Bizio pers. comm.), often with presence of *Cistus* bushes.

Additional specimens examined. **Austria**, Kärnten, Bezirk Spittal an der Drau, Reißleck, Penk, alt. 1100 m, under *Picea abies*, 16 Aug. 2013, D. Bandini & B. Oertel (KR-M-004315); Salzburg, Bezirk Tamsweg, Tamsweg, near Pretzenes, alt. 1500 m, in *Picea abies* forest, 15 Aug. 2013, D. Bandini & B. Oertel (KR-M-004316); Tirol, Bezirk Landeck, Fließ, Pillerhöhe, alt. 1540 m, in *Picea abies* forest, 28 Aug. 2014, B. Oertel & G. Schmidt-Stoehr (KR-M-0004313). — **Finland**, Enontökö-Lappi, Enontekiö, Kilpisjärvi, along the small river Tšahkaljohka, in rather moist forest of *Betula pubescens* subsp. czerpanovii, 15 Aug. 1990, J. Vauras (TUR-A 145165, duplicate in KR-M-00046553); Koillismaa, Kuusamo, Oulanka National Park, near the Research Station, mixed forest with *Pinus sylvestris*, *Picea abies* and *Betula sp.* sp., 17 Aug. 2015, J. Vauras, D. Bandini & B. Oertel (KR-M-0046554); Kuusamo, Oulanka National Park, foot of Ampumavara, roadside with *Pinus sylvestris*, *Picea abies* and *Betula sp.*, 20 Aug. 2015, J. Vauras, D. Bandini & B. Oertel (KR-M-0046555); Kuusamo, Oulanka National Park,铍hlännä, wayside with *Pinus sylvestris*, *Picea abies* and *Betula sp.*, 21 Aug. 2015, J. Vauras, D. Bandini & B. Oertel (KR-M-0046556); Kuusamo, Oulanka National Park, foot of Ampumavara, roadside with *Pinus sylvestris*, *Picea abies* and *Betula sp.*, 22 Aug. 2015, J. Vauras, D. Bandini & B. Oertel (KR-M-0046557); Kuusamo, Oulanka National Park, Liikasenvaara, lawn of a house of Iso Sirkkalampi, in mixed forest.
I. mixtilis

Moreno (2009), as 36148, duplicate in AC 3189; published by Esteve-Raventós & Caballero

Quercus ilex

subsp. (AH 40279, duplicate in AC 4102); Villarroya, alt. 800 m, in decalcified sandy

(AH 36110, duplicate in AC 2008); 6 June 2009

J. Muñoz

Nov. 2014,

Cáceres, Jarandilla de la Vera, in acid soil under

La Roca de l’Estany, alt. 2150 m, under

slightly calcareous soils, 2 Aug. 1997,
duplicate in JVG 970812-8); 23 July 1999,

J. Vila & X. Llimona

among

Queralbs, Val de Núria, Coma de l’Embut, alt. 2220 m, in calcareous soil

(AH 30697); Cataluña, Gerona,

(AH 24989); Valladolid, Tudela de Duero, in slightly acid and sandy soil in

León, Burgos, Valle de Mena, Vivanco, Santuario de Cantonad, alt. 480 m,

J.M. Barrasa & F. Esteve-Raventós

Ordunte, alt. 300 m, under

in slightly acid and sandy soils,

Quercus robur

Moreno

(AH 40499); Castilla-León, Burgos, Nava de Mena, Embalse de

Ordunte, alt. 300 m, under Quercus robur in slightly acid and sandy soils,

14 Oct. 1998, J.M. Barrasa & F. Esteve-Raventós (AH 24987); Castilla-León,

Burgos, Valle de Mena, Vivanco, Santuario de Cantonal, alt. 480 m,

in Fagus sylvatica forest, 14 Oct. 1998, J.M. Barrasa & F. Esteve-Raventós (AH 49899); Valladolid, Tudela de Duero, in slightly acid and sandy soil in

Pinus pinea forest, 23 Nov. 2002, P. Juste (AH 30697); Cataluña, Gerona,

Queraltas, Val de Núria, Coma de l’Embut, alt. 2220 m, in calcareous soil

among Dryas octopetala and Salix retusa, 8 Aug. 1996, J. Vila (AH 21573,
duplicate in JVG 960808-26); 12 Aug. 1997, J. Vila & L. Llimona (AH 23481,
duplicate in JVG 970812-8); 23 July 1999, J. Vila (AH 26731, duplicate in JVG 990723-15; published by Esteve-Raventós & Vila (1997), as I. mixtilis); Gerona, Seitzcases, Refugio de Ullèter, alt. 2300 m, among Salix retusa in slightly calcareous soil, 14 Aug. 1997, J. Vila (AH 23480, duplicate in JVG 970802-4); Lérida, Espoy, Estany de Sant Maurici, La Roca de l’Estany, alt. 2150 m, under Salix pyrenaica or Salix sylvestris, 12 Sept. 1996, J. Vila & L. Llimona (AH 21576, duplicate in JVG 960912-25; published by Esteve-Raventós & Vila (1997), as I. mixtilis); Extremadura, Cáceres, Jarandilla de la Vera, in acid soil under Quercus pyrenaica, 14 Nov. 2014, J. Muñoz (AH 44531); La Rioja, Santa Lucía, Valle de Ochón, alt. 800 m, in decalcified sandy soil in Quercus ilex subsp. ballota forest, 3 June 1997, A. Caballero (AH 36110, duplicate in AC 2008); 6 June 2009

(AH 40279, duplicate in AC 4102); Villarroya, alt. 800 m, in decalcified sandy soils of Quercus ilex subsp. ballota forest, 30 April 2006, A. Caballero (AH 36148, duplicate in AC 3189; published by Esteve-Raventós & Caballero Moreno (2009), as I. mixtilis).

Notes — This study suggests that I. occulta is the species with the broadest, transoceanic distribution within the Mixtilis group, and probably the most common one. Its epithet refers to the fact that this taxon has been overlooked and has probably been misinterpreted for I. mixtilis or other species of this group.

Inocybe occulta forms a well-supported clade in our analyses (Fig. 1) but it is sometimes difficult to recognize morphologically. Typical collections are characterized by the heterodiametrical spores, pleurocystidia 34–69 μm long and a pileus of intense

Typical collections are characterized by the heterodiametrical group, and probably the most common one. Its epithet refers to the broadest, transoceanic distribution within the Mixtilis group, and probably the most common one. Its epithet refers to

to those of I. nothomixtilis, I. johannis-stanglii and I. subtrivialis, which have considerably lower knobs.

Inocybe subtrivialis Esteve-Rav. , M. Villarreal & Heykoop, Rev. Catalana Micr. 20: 157. 1997 — Fig. 13, 14

Holotypus. Span, Castilla-La Mancha, Toledo, La Igliesuela, El Bonal, 30ÚTK525575, alt. 450 m, in acid sandy soil of Quercus ilex subsp. ballota forest with Cistus laurifolius and Lavandula pedunculata bushes, 16 Jan. 1996, J.L. Aguirre, M. Heykoop & M. Villarreal (AH 19225).

Selected descriptions and iconography: Esteve-Raventós et al. (1997: 157, f. 5). ITS barcode GenBank MH496013, MH496014.

Habitat & Distribution — Inocybe subtrivialis is currently known only in the Iberian Peninsula, but its ecology suggests it is more widespread and has been overlooked in Mediterranean Europe. Some records of I. pallida from Mediterranean environments might actually belong to I. subtrivialis, as that by Solak et al. (2009) in pine forests in southern Turkey. The typical habitat of I. subtrivialis is thermophilic Mediterranean evergreen oak forests (Quercus ilex subsp. ballota) or pine forests (Pinus pinea, P. pinaster), with presence of Cistus.

Specimens examined. Span, Toledo, La Igliesuela, El Bonal, 30ÚTK525575, alt. 450 m, in acid sandy soil of Quercus ilex subsp. ballota forest with Cistus laurifolius and Lavandula pedunculata bushes, 16 Jan. 1996, J.L. Aguirre, M. Heykoop & M. Villarreal (AH 26783); AH 26784. — Inocybe cf. subtrivialis. Span, Madrid, Rozas de Puerto Real, Embalse de los Morales, alt. 780 m, in acid sandy soil in a mixed forest with Pinus pinaster and Cistus laurifolius bushes, 14 May 2000, F. Esteve-Raventós & M. Villarreal (AH 26789).

Notes — Inocybe subtrivialis was described in detail by Esteve-Raventós & Caballero Moreno (2009), who commented on the similarities with I. pallida in the sense of Stangl (1989) (see comments under I. johannis-stanglii). Our ITS-RPB2 analyses (Fig. 1) revealed that both are close but independent species. Furthermore, both show a different distribution, being I. subtrivialis a characteristic thermophilic species associated with Mediterranean evergreen oaks (Quercus spp.), Cistus spp. and Pinus spp., in acid sandy soil (see also notes on I. johannis-stanglii for comparison).

Inocybe subtrivialis shows the following diagnostic characteristics: 1) pleurs covered by whitish veil in young specimens, usually sticky to viscid in wet conditions, with a warm brownish-yellow (Mu 10YR 5/6–5/8, 6/6–6/8), orange (Mu 7.5YR 6/6–6/8) to dark yellow-orange brown or ‘bronze’ colour (Mu 7.5YR 5/6–5/8, 4/6), smooth or often broken into appressed...
scales; 2) stipe in most cases with a napiform or subbulbous base, in some collections with a slightly marginate bulb, white and typically becoming fuscous yellow with age (especially evident in exsiccatas), densely pruinose all over but sometimes sparsely pruinose in the lower third; 3) lamellae without distinct violet or lilac shades when young; 4) spores heterodiametric, 
\[(8–)8.2–10.5–12.7(–12.8) \times (5.2–)5.5–6.4–7.3(–7.5) \mu m, Q = (1.25–)1.3–1.65–2(–2.2) \] [n = 100, from 4 collections], provided with numerous (10–)12–16(17) low and obtuse knobs (0.35–0.75 \mu m high, 0.7–2 \mu m broad at base), but in some occasional collections without distinct knobs and hence sub-angulose in outline; 5) pleurocystidia mostly narrowly lageniform to subfusiform, also sublageniform provided with a differentiated obtuse neck and base often tapering into a pedicel, 46–55.5–65 \times 9.5–16–22 \mu m [n = 40], walls (1–)1.2–2(–2.5) \mu m thick, yellowish (not very intense) in ammonia solutions, content often showing a pale yellow-fuscous intracellular pigment; and 6) caulocystidia similar to hymenial cystidia, often more slender or even hair-like near the base, mixed with clavate to sphaeropedunculate or subovoid paracystidia.

Specimen AH 26789 is morphologically similar to *I. subtrivialis* but its ITS sequence deviates from the one obtained from the holotype specimen (11 changes and several insertions/deletions). Our material needs to be examined to more appropriately assess its identity. In addition to its habitat and distribution, *I. subtrivialis* differs from *I. johannis-stanglii* in the darker, brownish orange pileal colour, a strong tendency of the fruitbodies to become yellow fuscous to bronze on ageing or upon drying, slightly narrower spores and slenderer cystidia.
I. johannis-stanglii
I. nothomixtilis
I. ceskae
I. subtrivialis

DISCUSSION

Inocybe is a species-rich genus of ectomycorrhizal fungi. Due to lack of reference sequences and lack of comparative taxonomic studies, identification of *Inocybe* species is often challenging and many sequences and material remain unidentified or misidentified (Ryberg et al. 2008). Previous ITS-based phylogenies integrating comparative morphological studies and distributional data have proven to be a good approach to resolve several species complexes (e.g., Larsson et al. 2009, 2017, Cripps et al. 2010, Esteve-Raventós et al. 2015, 2016) and six species are recognized here within the *I. mixtilis* group. The addition of RPB2 sequences to the ITS data yielded a more robust phylogeny, especially concerning deeper nodes. Our analyses confirmed also that a considerable amount of sequences remain being erroneously identified in GenBank (Ryberg et al. 2008). Thus, sequences of at least *I. ceskae* and *I. occulta* have been named as *I. mixtilis* in GenBank (Table 1).

The six species recognized in this study are rather well delimited using morphological, ecological and distributional characters. Among morphological characters, spore shape and cystidial length/shape, pileus colour and viscosity and the presence of velipellis were useful in species identification, but some characters overlap between close species. Thus, *I. mixtilis*, *I. ceskae* and *I. occulta*, distinct species according to our phylogenetic analyses (Fig. 1), cannot always be reliably identified through morphological species recognition and we consider them ‘semicryptic’ species. From a molecular point of view, these taxa are regarded as distinct phyllospecies (Wilkins 2009), constituting three diagnosable, monophyletic entities that represent unique lineages in the complex. The term semicryptic has been applied to species subsumed under a collective epithet and having overlapping morphological characters (Bickford et al. 2006), as in the *I. mixtilis* group and also used in similar cases concerning some ‘telamonioid’ *Cortinarius* groups by Brandrud et al. (2015).

Interestingly, our phylogenetic analyses (Fig. 2) show certain patterns in host-specificity and distribution, but conclusions should be taken cautiously as our sampling includes a small representation of the potential diversity of the *I. mixtilis* group. Two species, *I. ceskae* and *I. occulta* have a transoceanic distribution, the former being apparently restricted to subboreal and boreal forests. On the contrary, *I. mixtilis* is so far only known in Europe and Asia, but examination of further specimens is needed to confirm this. In general, species of the *I. mixtilis* group seem to have a broad host-specificity since all species but *I. johannis-stanglii* seem to establish ectomycorrhizal association with both conifers and angiosperms, as earlier observed for other groups of *Inocybe* (Ryberg et al. 2011, Esteve-Raventós et al. 2016).

This study represents one more example of cryptic speciation in *Inocybe*. The use of the ITS region and comparative morphological studies were useful to disentangle the *I. mixtilis* complex also in this case and allowed us to make a step further towards a better understanding of this ecologically important and diverse genus of ectomycorrhizal fungi.

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