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Porpoloma aranzadii is a synonym of *Mycena dura* further notes in *Mycena* sect. *Calodontes*

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Abstract – *Mycena* sect. *Calodontes* is a taxonomically intricate group comprising phylospecies so far not recognizable using a morphological recognition approach. In this study we re-examine and sequence Iberian material of *Porpoloma aranzadii*, including the type specimen. The dextrinoid lamellar trama and mycelial hyphae in the type specimen, the latter covered with crystals, suggest a placement in *Mycena* sect. *Calodontes*. The type specimen of *M. pura* f. *brunnea* is also examined and sequenced. We constructed an ITS dataset representing all European phylospecies reported in previous studies, and we coded indel information. Our phylogenetic analyses recovered strongly supported clades for all the phylospecies and indel information provides additional resolution in phylospecies delimitation. *Porpoloma aranzadii* sequences are encompassed in the *M. dura* clade and their synonymy is proposed. Further information on the macroscopic variability of *M. dura* is obtained. Within sect. *Calodontes, M. dura* is characterized by the fascicled or caespitose basidiomata, a grassland habitat and sometimes the lack of any raphanoid odour. Its distribution is extended to the Iberian Peninsula. The sequence obtained from the type of *M. pura* f. *brunnea* nests in *M. pura* clade 9. The new combination *M. brunnea* is proposed to formally refer to this clade at species rank. *Mycena brunnea* appears to be characterized by a predominantly brown pileus, the absence of deep purple tones and a forest habitat. *Mycena brunnea* is also macroscopically reminiscent of *M. dura*, but the former differs mainly in a gregarious growth of basidiomata and a forest habitat.

Species delimitation / indel coding / *Prunulus* / ITS

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INTRODUCTION

Mycena sect. *Calodontes* (Fr.: Fr.) Quél. sensu Maas Geesteranus (1989) is a group of agaricoid basidiomycetes. It is characterized by having collybioid basidiomata, often with purple tones, voluminous cheilocystidia, often amyloid spores and a saprobic trophic strategy (Harder *et al*., 2010). Despite previous attempts proved unsuccessful (Boisselier-Dubayle *et al*., 1996), Chew *et al*. (2014) obtained for the first time cultures of species of sect. *Calodontes*. Microscopic features of rhizomorphs are characteristic due to the presence of dextrinoid hyphae and to being covered with abundant crystals (Clémençon, 2004). This has not been tested, however, across all members of sect. *Calodontes*. Some species have recently been discovered to be bioluminiscent (Chew *et al*., 2014). The generic name *Prunulus* S.F. Gray has sometimes been used for *Mycena* sect. *Calodontes* (Redhead *et al*., 2001), but conclusive molecular evidence supporting the monophyly of sect. *Calodontes* is missing. Thus, a conservative approach of continuing the usage of sect. *Calodontes* is adopted here, as done before (Harder *et al.*, 2010; 2013).

Species delimitation in sect. *Calodontes* is problematic. Whereas a few taxa have clear morphological taxonomic boundaries (e.g. *Mycena rosea* Gramberg or *M. pelianthina* (Fr.: Fr.) Quél.), *Mycena pura* (Pers.: Fr.) P. Kumm. has been considered to be a morphologically and chromatically extremely variable species, many infraspecific names being coined to reflect this morphological variability (Maas Geesteranus & Hausknecht, 1994; Robich, 2003). Harder *et al.* (2010) used ITS molecular data to evaluate species limits in sect. *Calodontes* and concluded that there is correspondence between the conventional morphological species recognition and the signal obtained from the ITS region in all taxa but in *M. diosma* and *M. pura*. In total, Harder *et al.* (2010) detected ten cryptic phylogenetic lineages within *M. pura* using the ITS region, but were not able to find morphological traits characterizing each of the lineages. The basidioma colour, previously used as diagnostic character when separating infraspecific taxa within *M. pura*, was found to be very variable even within a phylospecies and therefore to lack taxonomic value for species delimitation within the *M. pura* complex.

A second molecular study by Harder *et al.* (2013) assessed the adequacy of the ITS region using two more protein coding genes (RPB1 and tEF-1∂). These authors observed recombination events between ITS1 and ITS2, most markedly in the phylospecies 9. Despite the limitations of the ITS for species recognition in sect. *Calodontes* were stressed in this study, separate analyses of RPB1, tEF-1∂ and ITS showed a high congruence in *Mycena pelianthina, M. diosma, M. rosea, M. dura, M. lammiensis & M. pearsoniana*. In total this study retrieved 11 phylospecies within the *M. pura* complex.

Porpoloma aranzadii Laskibar, Arrillaga & M. Bon was described from the North of the Iberian Peninsula as the only European *Porpoloma* having voluminous cheilocystidia (Laskibar *et al.*, 2001). The white spore print, presence of clamps and amyloid smooth spores were the key characters for its generic placement. *Porpoloma aranzadii* was collected in a calcareous pasture, having a pale beige to ochre brown cap and "sweet and slightly aromatic" smell. The lamellar trama was described as dextrinoid, but this character was not granted taxonomic importance. The genus *Porpoloma* has been recently segregated into several smaller genera (Sánchez-García *et al.,* 2014).

The examination of additional specimens of *P. aranzadii*, collected mainly in the type locality, having a weak raphanoid smell and dextrinoid lamellar trama hyphae, has led to the idea that *P. aranzadii* is connected to *Mycena* sect. *Calodontes*.

Also, rhizomorph microscopic features conform to the description of rhizomorphs of sect. *Calodontes* provided by Clemençon (2004, as *Prunulus*). The main aim of this study is to evaluate the phylogenetic placement of *P. aranzadii* using ITS molecular data and to examine again the type material in the context of its affinities with sect. *Calodontes*. Likewise, the type of *Mycena pura* f. *brunnea* is examined and sequenced due to a possible relationship with *P. aranzadii*.

MATERIALS AND METHODS

Morphological study: The description is based on fresh collections and dried herbarium specimens provided with colour photographs. Colour codes are based on Kornerup & Wanscher (1961). Congo red in ammonia or SDS, Melzer´s reagent, phloxine, NH_4OH 15% and KOH 5% were used for microscopical examination. Spore statistics were calculated following Heinemann & Rammeloo (1985) and based on measures of 20 spores per collection from lamellar sections rehydrated in KOH 5%. Abbreviations of statistics referring to basidiospores are: L_m = mean length; W_m = mean width, $Q_m = L_m/W_m$. Material is deposited in ARAN and M herbaria (Thiers 2014).

DNA extraction, PCR amplification and sequencing: DNA was extracted from dried specimens. Total genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and following the manufacturer´s instructions. For the holotype specimen of *Porpoloma aranzadii*, the extraction method followed Hansen *et al.* (1999). Material was ground using a plastic pestle after adding a few sand particles, in eppendorf tubes. The entire ITS1-5.8S-ITS2 region was sequenced using the primers ITS1-ITS4 (White *et al.*, 1990). For the type specimens of *Porpoloma aranzadii* and *Mycena pura* f. *brunnea* the ITS1 and ITS2 regions were amplified in two pieces using the primers ITS1-ITS2 and ITS3-ITS4, respectively. PCR reactions were performed as follows: 95ºC hot start for 5 min, followed by 35 cycles of 45, 30, and 45 sec. at 94ºC, 55ºC and 72ºC respectively, with a final step at 72ºC for 10 min. Annealing temperature was lowered and the amounts of cycles raised when working with problematic samples. PCR products were purified using ExoSAP-IT® (USB, Cleveland, OH, USA). The amplicons were sequenced by Macrogen Europe (The Netherlands).

Novel sequences generated in this study were subjected to BLAST to screen for contaminants and submitted to the MBL/GenBank/DDBJ databases (Cochrane *et al.*, 2010). Sequences were assembled and edited using Sequencher 4.7 software (Gene Codes, Ann Arbor, Michigan, USA), and aligned with further ITS sequences of *Mycena* sect. *Calodontes* downloaded from GenBank (Table 1). Taxon sampling is identical to the one used in Harder *et al.* (2013), excluding BAP132 and TL9433 and adding our newly generated sequences. Sequences were aligned employing the alignment tool in AliView (Larsson, 2014) and then optimized manually.

Phylogenetic analyses: Specimens sequenced in this study are presented in Table 1. A sequence of *Mycena rubromarginata* (FN394624) was selected as outgroup, as done by Harder *et al.* (2010). The alignment was analysed using Bayesian (MB) and Maximum Likelihood (ML) approaches. For the Bayesian analysis, indel information was coded in a second binary data partition in SeqState (Müller, 2005), following the simple coding method by Simmons & Ochoterena (2000). The Bayesian analysis was conducted in MrBayes v. 3.2.1. (Ronquist *et al.*, 2012). In the first

Table 1. Voucher specimens used in the phylogenetic analyses, together with their GenBank Accession number. Type specimens are in bold. *M.* = *Mycena, P.* = *Porpoloma*

Original identification	*Clade*	*Voucher specimen*	*GenBank Accession no*
M. pura* f. *brunnea	***M. brunnea*** (*M. pura* 11)	M-0160144	**KT222187**
M. pura	*M. brunnea* (*M. pura* 11)	CBH386	FN394565
M. pura	*M. brunnea* (*M. pura* 11)	CBH187	FN394564
M. diosma	*M. diosma*	LK1191	FN394619
M. diosma	*M. diosma*	CBH400	FN394617
M. dura	***M. dura***	**WU 10315**	FN394560
P. aranzadii	***M. dura***	**ARAN-Fungi 2950**	**KT222188**
P. aranzadii	*M. dura*	ARAN-Fungi 1387	**KT222189**
P. aranzadii	*M. dura*	ARAN-Fungi 1966	**KT222190**
M. lamniensis	*M. rosea*	TUR165927	FN394552
M. luteovariegata	***M. luteovariegata***	**CBH226**	FN394604
M. pura f. *lutea*	*M. luteovariegata*	DB2005/152	FN394603
M. pura	*M. luteovariegata*	TL5614	FN394602
M. pearsoniana	*M. pearsoniana*	CBH068	FN394614
M. pearsoniana	*M. pearsoniana*	JV06890	FN394612
M. pearsoniana	*M. pearsoniana*	LK8802002	FN394613
M. pelianthina	*M. pelianthina*	CBH016	FN394547
M. pelianthina	*M. pelianthina*	CBH015	FN394549
M. pura	*M. pura* 1	CBH039	FN394588
M. pura	*M. pura* 2	CBH404	FN394566
M. pura	*M. pura* 2	CBH169	FN394579
M. pura	*M. pura* 2	CBH105	FN394581
M. pura	*M. pura* 2	CBH366	FN394572
M. pura	*M. pura* 3	CBH019	FN394605
M. pura	*M. pura* 3	CBH022	FN394574
M. pura	*M. pura* 3	KK	FN394606
M. pura	*M. pura* 4	JV06979	FN394585
M. pura f. *alba*	*M. pura* 4	CBH410	FN394595
M. pura f. *multicolor*	*M. pura* 4	TL4571	FN394583
M. pura	*M. pura* 4	TL12786	FN394591
M. pura	*M. pura* 7	IS10/11/2000	FN394611
M. pura	*M. pura* 8	CBH216	FN394598
M. pura	*M. pura* 8	CBH402	FN394599
M. pura	*M. pura* 9	CBH166	FN394607
M. pura	*M. pura* 9	CBH358	FN394608
M. pura	*M. pura* 9	CBH371	KF913023
M. pura	*M. pura* 9	CBH367	KF913022
M. pura f. *violacea*	*M. pura* 10	BAP165A	FN394563

Table 1. Voucher specimens used in the phylogenetic analyses, together with their GenBank Accession number. Type specimens are in bold. *M.* = *Mycena, P.* = *Porpoloma (continued)*

Original identification	*Clade*	*Voucher specimen*	*GenBank Accession no*
M. rosea	*M. rosea*	UP2	FN394550
*M. rosea_*	*M. rosea*	CBH383	FN394553
M. rosea	*M. rosea*	CBH097	FN394556
M. rosea	*M. rosea*	TL12409	FN394557
M. rosea	*M. rosea*	TL12393	FN394555
M. rosea	*M. rosea*	CBH409	FN394551
M. aff. *pura*	*M. spp.* (Ecuador)	TL9433	FN394622
M. sp.	*M. spp.* (Ecuador)	TL8052	FN394623
M. sp.	*M. spp.* (Ecuador)	TL9678	FN394621
M. rubromarginata		JV09362	FN394624

nucleotide partition, the substitution model was sampled across the GTR space by the 4 MCMC analyses. For the second partition, an F-81-like model was chosen and the "lset" parametre was set to "noabsencesites". Two parallel analyses of four MCMC chains were run for 15^6 generations, starting from a random tree, and sampling one tree every 100^{th} generation. To check if the chains had converged, determine if the mixing was adequate and to choose an appropriate burnin, log-likelihood values were plotted against the time generation using Tracer v. 1.5 (Rambaut & Drummond, 2007). Stationarity was assumed when average standard deviation of split frequencies fell below 0.01. A burnin sample of 75 000 trees was discarded from each run. To assess branch confidence, a 50% majority rule consensus tree was computed with the remaining 150 002 trees using the sumt command of MrBayes. Bayesian PP values $\geq 95\%$ were considered to be significant. The ML analysis was implemented via CIPRES Science Gateway (Miller *et al.*, 2010), employing the "RAxML HPC2 on XSEDE" tool (Stamatakis 2006), using mixed models of evolution, starting from a random tree and leaving the remaining options as default. For branch confidence, 1000 ML bootstrap replicates were conducted, using rapid bootstraping. A 50% majority rule consensus tree was made to obtain the bootstrap values in PAUP 4.0 Beta for Mac (Swofford, 2002). ML bootstrap values were placed on the majority rule Bayesian phylogram. The alignment and the 50% majority rule consensus tree of the Bayesian analysis are available in TreeBASE under the accession number S17843.

RESULTS

Four new sequences were generated in this study (Table 1) and were aligned with 44 sequences downloaded from GenBank. The simple coding system yielded a binary dataset of 70 characters. The complete alignment including indel information had 719 characters (nucleotide data 1-649; indel data 650-719). The Bayesian analysis reached an average standard deviation of split frequencies of 0.01 after 1 645 000 generations. The 50% majority rule consensus tree obtained from the MB

Fig. 1. 50% majority rule consensus Bayesian phylogram of the ITS region in *Mycena* sect. *Calodontes*, including coded indel information. Posterior Probabilities (≥ 95%)/Maximum Likelihood bootstrap values (≥ 70%) shown by each node. Thickened branches indicate that the node is supported in Bayesian and/or Maximum Likelihood analyses. Sequences from type specimens are in bold. Genus abbreviations: M=*Mycena*, P=*Porpoloma*.

analysis is presented in Fig. 1. The ML analysis resulted in a single best ML tree of -lnL = 2900.686145. The supported topology (PP ≥ 95%; ML ≥ 75%) was similar in MB and ML analyses.

Thirteen clades are strongly supported at least in one of the analyses. *Mycena* spp. (Ecuador) clade shows a clear internal genetic divergence. Sequences identified as *Porpoloma aranzadii* form a strongly supported clade (PP and ML 100%), together with a sequence from the holotype of *Mycena dura*. We therefore

Fig. 2. Macroscopic variability in *Mycena dura*. **a.** basidiomata with a purple hue (ARAN-Fungi 1076; **b.** more fleshy basidiomata with dark pileus, fascicled (ARAN-Fungi 1073); **c.** basidiomata with dark pileus (ARAN-Fungi 1075); **d.** strongly fascicled basidiomata, showing lamellae ranging from greyish white to pale pink (ARAN-Fungi 1387). Photographs: Pedro Arrillaga (a, b, c), Adrián Hereza (d).

propose *Porpoloma aranzadii* as a synonym of *Mycena dura*. The sequence obtained from the holotype of *M. pura* f. *brunnea* is supported with two more sequences (PP ≥ 100%; ML ≥ 96%; clade *M. pura* 5 in Harder *et al.* (2010)).

TAXONOMY

Mycena dura Maas Gest. & Hauskn., *Österr. Z. Pilzk.* 3: 5. 1994. **Figs 2, 3**

Holotype: AUSTRIA. Burgenland. Trausdorf prope Eisenstadt, 20 Oct. 1991, leg. U. Passauer (988.279-754, L). *Isotype* WU 10315. GenBank barcode: FN394560.

= *Porpoloma aranzadii* Laskibar, Arrillaga & Bon, *Doc. Mycol.* 30(120): 49. 2001.

Holotype: SPAIN. Burgos. Galbarros, 28 May 2000, leg. P. Sainz, ARAN-Fungi 2950 (!). GenBank barcode: KT222188.

Basidiomata gregarious or caespitose, growing in rows or rings. *Pileus* 20-45(60) mm diam., not fleshy, initially obtusely conical or campanulate, then convex with a plane centre, broadly umbonate. Margin sometimes irregular. Cuticle hygrophanous, drying out from centre outwards, glabrous or slightly innately fibrillose, sometimes slightly wrinkled near the margin, translucently striate when

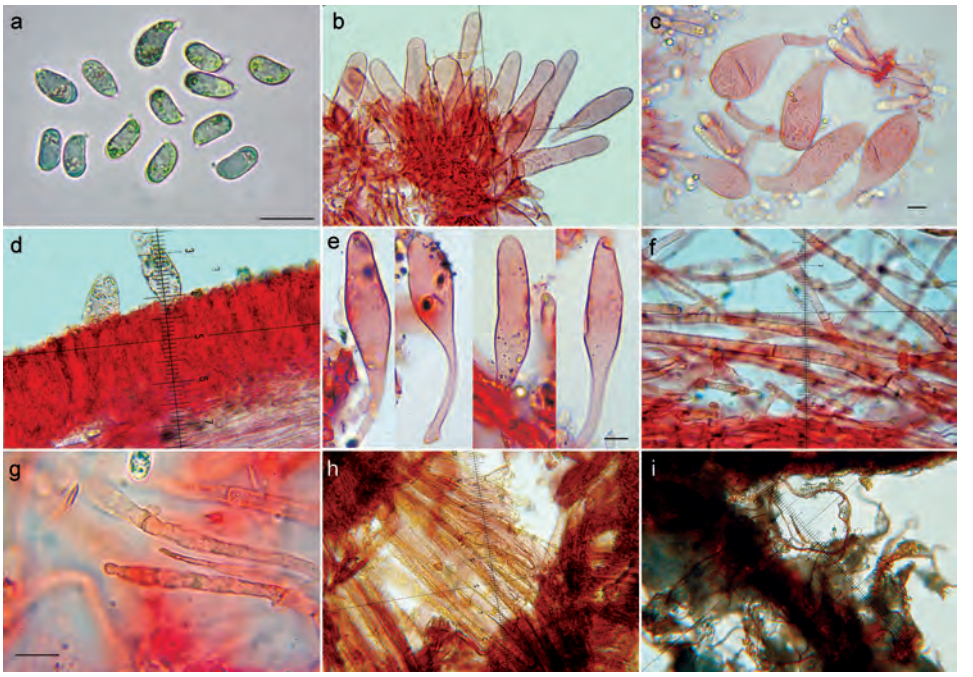

Fig. 3. Microscopic characters in *Mycena dura*. **a.** basidiospores in Melzer´s reagent (ARAN-Fungi 1387); **b.** cheilocystidia in bundles/clusters (ARAN-Fungi 1076); **c.** cheilocystidia showing a crackled surface (ARAN-Fungi 1387); **d.** pleurocystidia protrudring from the hymenium (ARAN-Fungi 2950); **e.** pleurocystidia (ARAN-Fungi 1387); **f.** pileipellis hyphae (ARAN-Fungi 1076); **g.** terminal hypha of pileipellis and clamp connection; **h.** dextrinoid hyphae in the lamellar trama (ARAN-Fungi 2950); **i.** dextrinoid mycelial hyphae covered with crystals (ARAN-Fungi 2950). Scale bar 10 µm; in photos with measuring grid 10 divisions = 25 µm (fig. d, h, i) or 10 µm (b, f). Photographs Pedro Arrillaga, except for c, e and g (Adrián Hereza).

moist, sometimes shortly sulcate. Colour initially ochre brown (6C5-6), orange brown (6AC-D), sometimes dark brown (9F5) or with purple hue (9E5), then paler, pale greyish brown (6A3), yellowish beige (5B5), ochraceous brown (6B3-4), sometimes with purple or reddish purple tones (7C6). **Lamellae** initially adnate, then emarginate with a decurrent tooth or not, ventricose to subtrapezoidal in side view. Colour initially pale pink (7A2), then greyish white to white (7A1). Gill-edge regular or somewhat sinuous in mature specimens. **Stipe** 30-60(80) × 5-12(15) mm, cylindrical, sometimes compressed or distorted, full or fistulose, longitudinally fibrillose, glabrous or very finely puberulous, especially at the apex, rarely pubescent at apex. Colour white (7A1) to greyish white (between 7A1-7B1), sometimes very pale beige (6A2). White rhizomorphs present at the base, branched. **Context** thin in pileus, 1-2(3) mm thick, fragile, white, cream white. Odour complex and variable, in some collections weakly raphanoid with aromatic hue, then more or less herbaceous, aromatic. Taste weakly bitter and slightly farinaceous. Spore print white.

Basidiospores ellipsoid, sometimes subamygdaliform, smooth, amyloid, 6.2-9 × 4.2-5.2 µm (L_m = 7.4-7.7; W_m = 4.2-4.3; Q_m = 1.7-1.8). **Basidia** narrowly clavate, 4-spored, 30-45 × 6-9 µm. Gill edge heteromorphic. **Cheilocystidia** rather

abundant, clustered, variable in shape, broadly clavate, fusiform or lageniform, apex rostrate or lanceolate, sometimes with crackled surface, 40-90 (110) × (12)15-20(28) µm. **Pleurocystidia** scarce, generally broadly clavate to subcylindrical, of similar size as cheilocystidia. Lamellar trama regular, formed by 5-15 µm broad hyphae, dextrinoid. **Caulocystidia** subcylindrical to narrowly clavate, 37-75 × 8-15(20) µm. Pileipellis of a cutis, hyphae 1.5-6(7) µm broad, with mixed intracellular and parietal pigment, dextrinoid. **Rhizomorphs** without cortex, composed of strongly dextrinoid hyphae, covered by crystals. Clamp connections abundant.

Examined material: SPAIN. Burgos: Galbarros, 30TVN6408, 992 m a.s.l., on grassy ground in calcareous pasture, 28 May 2000, P. Sainz, ARAN-Fungi 2950 (holotype); 4 May 1993, ARAN-Fungi 1071; 11 May 1993, ARAN-Fungi 1072; 4 May 2003, ARAN-Fungi 1073; 11 May 2003, ARAN-Fungi 1074; 17 May 2008, ARAN-Fungi 1075; 1 June 2008, ARAN-Fungi 1076; ARAN-Fungi 1966. Soria: Valdelinares, pasture on calcareous ground, 13 June 2013, A. Hereza, ARAN-Fungi 1387.

Commentary: The re-examination of the type specimen of *Porpoloma aranzadii* confirmed its conspecificity with *M. dura* macro- and micromorphologically. The rhizorphs of the holotype of *P. aranzadii* showed strongly dextrinoid hyphae covered with numerous crystals (Fig. 3 i). Although the original description of *P. aranzadii* did not mention pleurocystidia, rare pleurocystidia were found in the type specimen (Fig. 3d). Scarce pleurocystidia were also seen in the type specimen of *Mycena dura* (Maas Geesteranus & Hausknecht, 1994).

Mycena dura was hitherto known from the type locality and a later collection by Hausknecht from Austria (WU 18414). It has also been recorded from Italy (Robich, 2003) and Northern Spain (Picón, 2008). Our new finds allowed us to obtain a better insight into the morphological variability of *M. dura*. They also extend the distribution of *M. dura* to the Iberian Peninsula. It seems reasonable to think that *M. dura* is present in many more localities where it has been overlooked.

As in other species of sect. *Calodontes, M. dura* shows a high variability in terms of pileus and lamella colour but, remarkably, specimens with deep purple or pink tones are unknown. Instead, the typical pileus colouration is ochre brown and thus uncommon within sect. *Calodontes*. The colour of the photo of the holotype of *M. dura* is darker than typically, according to the material seen by us. Nevertheless, the original description covers the colour variation observed in the Iberian material. Another remarkable feature of *M. dura,* in the context of sect. *Calodontes,* is the weakly raphanoid or non-raphanoid odour (Laskibar *et al.*, 2001; and personal observations), whereas all the species in the group but *M. diosma* have a strong and constant raphanoid odour (Harder *et al.*, 2010). By contrast, the protologue of *M. dura* describes the odour as "very much resembling" that of *M. pura*. Despite being inconstant, the absence of raphanoid smell is uncommon in sect. *Calodontes* (Harder *et al.*, 2010). The lamella edge is heteromorphic in *M. dura*, and cheilocystidia occur typically in clusters (Fig. 3b). Maas Geesteranus (1989) described the cheilocystidia as "forming a sterile band" in the lamella edge for several species within this complex. This can be another character to enable species recognition in sect. *Calodontes*, but here we have not checked it across all the phylospecies in sect. *Calodontes*. Nevertheless, all the *M. dura* material we have examined had a heteromerous lamella edge. Another observation of possible interest is that the surface of cheilocystidia was crackled in several specimens of *M. dura*, at least when observed in Congo red (Fig. 3b, 3c). Maas Geesteranus (1989) described cheilocystidia as "generally smooth" in the general description of sect. *Calodontes*, but described cheilocystidia as smooth in all species in which he described cheilocystidia. This

character must also be checked in further specimens to evaluate its taxonomic significance.

Harder *et al.* (2010) suggested that species in sect. *Calodontes* do not show any level of ecological specialization, and that specimens of most ITS groups had been collected in a *Fagus/Quercus* dominated forest. In contradiction with this view, *Mycena luteovariegata* was elevated to species rank with strong emphasis on its ecology in grassland habitats (Harder *et al.*, 2012). More information on the ecological preference of *M. dura* is made available in this study. All the specimens in the *M. dura* clade were collected in grassland habitats (Maas Geesteranus & Hausknecht, 1994; Laskibar *et al.*, 2001) and this is here proposed to be a key character of *M. dura*. The two Iberian localities are on calcareous bedrock. The material of *M. dura* reported by Robich (2003) is likely to represent another taxon within sect. *Calodontes,* due to its forest habitat. We therefore regard those records as doubtful.

Mycena brunnea (E. Ludw.) Olariaga, Pérez-De-Greg. & Arrillaga comb. nov. MycoBank MBT812957.

Basionym: *Mycena pura* f. *brunnea* E. Ludw., *Pilzkonpendium* 3: 711. 2012.

Holotype: SWEDEN. Skåne. Ivö, on calcareous ground under *Betula*, *Sorbus*, *Corylus*, leg. E. Ludwig, 29 Aug. 2008, leg. Ludwig 3637 (M-0160144) (!). GenBank barcode: KT222187.

Commentary: This taxon was described based on the brown colour and the absence of violaceous tones even in young basidiomata (Ludwig, 2012). The sequence from the type specimen nests together with two specimens analyzed in previous studies (Harder *et al.*, 2010 (*M. pura* 5); 2013 (*M. pura* 11)), namely CBH187 and CBH386. Although Harder *et al.* (2013) claimed that the ITS sometimes provides a misleading phylogenetic signal in the *M. pura* group, these two specimens formed consistently a strongly supported clade in separate RPB1, tEF-1∂ and ITS phylogenies and it can be asserted beyond doubt that the type of *M. pura* f. *brunnea* belongs to *M. pura* clade 11 in Harder *et al.* (2013). We accordingly elevate *M. pura* f. *brunnea* to species rank in order to make it available to formally refer to *M. pura* clade 11.

Specimens CBH187 and CBH386 were coded as having a "reddish" pileus, and "reddish" or "white" gills and stipe. Conspicuous violet tones appear to be absent in specimens of this clade. The original illustration of *M. pura* f. *brunnea* by Ludwig (2012), shows basidiomata highly reminiscent of *M. dura* due to a dark brown pileus and to the absence of remarkable violaceous tones. However, *M. brunnea* appears to differ from *M. dura* in being a forest-inhabiting species that grows gregariously and in having a strong raphanoid smell. Following this, the material reported by Robich (2003) under *M. dura* may actually represent *M. brunnea*. Future research is needed yet to evaluate whether *M. brunnea* can be identified through morphological species recognition alone.

DISCUSSION

The ITS region has been proposed as the universal DNA barcode marker for Fungi (Schoch *et al.*, 2012), but its phylogenetic signal in sect. *Calodontes* is not completely reliable according to Harder *et al.* (2013). These authors compared ITS, RPB1 and tEF-1∂ phylogenies of a very similar dataset to the one utilized by us and

determined which clades were not congruent across the three marker phylogenies. The *Mycena brunnea* and *M. dura* clades were congruent in the ITS, RPB1 and tEF-1∂ phylogenies. In this study, we introduce indel information in the analyses of the ITS region in the *M. pura* group. Indel information has rarely been considered in phylogenetic studies of fungi (Nagy *et al.*, 2012). In our dataset, inclusion of indel information resulted in higher support values, and all the phylospecies accepted in Harder *et al.* (2013) are supported in our ITS analyses.

Further contributions are needed to obtain a good understanding of species delimitation in sect. *Calodontes*. Our study contributes to clarify the taxonomic identity of two phylospecies within this group and reveals that *Porpoloma aranzadii* is a synonym of *M. dura*. Moreover, we confirm that the ecological information can be of aid in characterizing a few phylospecies, like *M. luteovariegata* (Harder *et al.*, 2013); also, *M. dura*, grassland inhabiting, and *M. brunnea*, forest inhabiting, appear to differ in their ecology as well.

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