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Stereoid genus *Heteroradulum* (*Basidiomycota*, *Auriculariales*) in the Czech Republic

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The current knowledge of the lignicolous genus *Heteroradulum* was clarified for the Czech Republic. The occurrences of both European species, *Heteroradulum deglubens* and *H. kmetii*, are based on field collections and herbarium specimens. *Heteroradulum deglubens* was collected on dead hardwoods in near-natural forests and its identity was confirmed by DNA sequences of the ITS region. *Heteroradulum kmetii* was collected in warmer parts of the Czech Republic only and differs from *H. deglubens* in its ecology. An extended host tree spectrum is confirmed for both species.

Key words: *Agaricomycetes*, *Auriculariaceae*, corticioid fungi, *Eichleriella deglubens*.

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Studie hodnotí současné poznatky o výskytu lignikolního rodu rozlitka (*Heteroradulum*) v České republice. Data o rozšíření obou evropských druhů, rozlitky osténkaté (*Heteroradulum deglubens*) a rozlitky Kmeťovy (*H. kmetii*), jsou založena na terénních sběrech a herbářových položkách. Rozlitka osténkatá byla sbírána na mrtvém dřevě listnáčů v zachovalých lesích a její identita byla potvrzena sekvencemi ITS úseku DNA. Rozlitka Kmeťova byla sbírána pouze v teplejších částech České republiky a od rozlitky osténkaté se liší ekologií. Pro obě rozlitky jsou potvrzeny nové hostitelské druhy dřevin.

INTRODUCTION

Fungi remain an unexplored group of heterotrophic organisms with a crucial ecological position among saprotrophic organisms in terrestrial ecosystems with woody plants. The corticoid and stereoid fungi of the *Auriculariales* (e.g. Weiß & Oberwinkler 2001, Li et al. 2023) serve as good examples of lesser-known taxa.

The group has not met with much interest from mycologists because it does not include any important wood pathogens. Malysheva & Spirin (2017) reported on the phylogeny and taxonomy of stereoid *Auriculariales* in detail, including genus *Heteroradulum* Lloyd ex Spirin & Malysheva. Species of *Heteroradulum* have longitudinally septate basidia with stalks and develop stereoid fruitbodies with pinkish, reddish or violet tints, which are also found in other stereoid species (e.g. in *Russulales*). Some light-coloured species of *Heteroradulum* were shifted to the new genus *Alloexidiopsis* L.W. Zhou & S.L. Liu, which lacks a pinkish colouration of the hymenophore, based mainly on DNA analyses of the internal transcribed spacer (ITS) and nuclear large subunit (nLSU) regions (Liu et al. 2022). In 2018, Yuan et al. (2018) described the genus *Grammatus* H.S. Yuan & Decock which is related to *Heteroradulum* but has a poroid-like hymenophore unusual in the *Auriculariales*. It was placed in synonymy with *Heteroradulum*. Currently, ten species have been accepted worldwide in *Heteroradulum* (Li et al. 2022, 2023). Most of the species are from tropical Asia with only two species known in Europe – the sister species *Heteroradulum deglubens* (Berk. & Broome) Spirin & Malysheva and *Heteroradulum kmetii* (Bres.) Spirin & Malysheva, which are the subject of this paper.

Although the two species are regarded well-studied in Europe, their current distribution and ecology are poorly known. This is because the name *Eichleriella deglubens* (Berk. & Broome) Lloyd was misapplied to the species *H. kmetii*, since young basidiocarps of the two species are macroscopically quite similar (Wells & Raitviir 1980, Malysheva & Spirin 2017). Microscopically, the species can be distinguished by their hyphal structure, which is monomitic in *H. deglubens* while dimitic in *H. kmetii* (Malysheva & Spirin 2017).

Malysheva & Spirin (2017) reported a wide distribution of *H. deglubens* in Europe but with few reports from Central Europe. One record of *H. deglubens* is confirmed by DNA from Germany (AF291272, Weiß & Oberwinkler 2001). Some records of *H. deglubens* are presented for Austria (GBIF, https://www.gbif.org/occurrence/search?taxon_key=9688507), some from the Czech Republic are deposited in Czech herbaria, but without confirmation based on DNA sequences to date. On the other hand, *H. kmetii* is widespread throughout the Holarctic realm (Malysheva & Spirin 2017).

This paper deals with the two *Heteroradulum* species reported from the Czech Republic. Morphological and molecular methods were used to identify and confirm newly collected and herbarium specimens of *H. deglubens* and *H. kmetii*, and to clarify and extend the knowledge of their distribution and ecology in the Czech Republic.

MATERIAL AND METHODS

Fresh specimens of *H. deglubens* were collected in West Bohemia, Czech Republic. They were deposited in the Museum of West Bohemia (PL) and duplicates in the herbarium of the University of West Bohemia. Revision was carried out for herbarium specimens from the Mycological Department of the National Museum, Prague (PRM), Moravian Museum, Brno (BRNM), Museum of Eastern Bohemia, Hradec Králové (HR), Oslo Museum, Norway (O), and some specimens were loaned by D. Dvořák (Masaryk University, Brno). Elevations for the records of herbarium specimens were taken from the herbarium data or estimated from maps. Precipitation totals in the month of specimen collection were taken from the nearest meteorological station.

We used the abbreviations of herbaria from Index Herbariorum (https://sweetgum.nybg.org/science/ih/), KBI for the herbarium of the Department of Biology, Geoscience and Environmental Education, Faculty of Education, University of West Bohemia, and D.D. for specimens of D. Dvořák. Other abbreviations used are NP for National Park, NNR for National Nature Reserve, NR for Nature Reserve, and NNM for National Nature Monument.

The features of *H. deglubens* and *H. kmetii* mentioned below are based on our observations of fresh and herbarium specimens collected in the Czech Republic. An Olympus SZ51 binocular stereomicroscope was used for macroscopic examinations, an Olympus BX51 optical microscope for microscopy, and an Olympus DP 72 camera for microscopic images. Microscopic preparations were made in Melzer's reagent and Cotton blue. Microscopic revision of the examined material was carried out by the authors, except specimens identified by A. Jirsa from the Moravian Karst and by L. Zíbarová from Litovelské Pomoraví.

The molecular procedure was performed according to Westphalen et al. (2018). The final ITS dataset included 15 sequences of which three were newly generated (GenBank: PQ877706, PQ877707, PQ877708), while the remainder were retrieved from Genbank (https://www.ncbi.nlm.nih.gov/genbank). Tab. 1 lists sequences of ITS nrDNA used in the phylogenetic analyses. Phylogenetic analyses were conducted using MEGA version 12 (Kumar et al. 2024). The marginal parts of sequences with gaps were trimmed, and the alignment was performed using MUSCLE with default settings in MEGA 12. The phylogeny was inferred using the Maximum Likelihood method and Kimura 2-parameter model of nucleotide substitutions (Kimura 1980). The evolutionary rate differences between sites were modelled using a discrete Gamma distribution across 5 categories (+*G*, parameter = 0.2715). The complete deletion option was applied to eliminate positions containing gaps and missing data, resulting in a final dataset comprising 370 positions. Phylogenetic analyses were conducted in MEGA 12 utilising up to 6 parallel computing threads.

The tree with the highest log likelihood (–1,194.99) is shown. Bootstrap percentages from 500 replicates are given next to the branches (Felsenstein 1985).

RESULTS AND DISCUSSION

We confirmed the occurrence of both European species of *Heteroradulum* (*H. deglubens*, *H. kmetii*) in the Czech Republic. Most of the studied specimens were stored in public herbaria as *Eichleriella deglubens*. *Heteroradulum deglubens* was confirmed from ten localities in the Czech Republic (six from Bohemia, four from Moravia). Two specimens from West Bohemia were verified by sequences of the ITS region. *Heteroradulum kmetii*, on the other hand, is found primarily in the south-eastern part of the country (South Moravian region),

Tab. 1. Used sequences of ITS nrDNA. Newly generated sequences are indicated in boldface.

Species	ITS GenBank accession numbers	Substrate	Country	Reference
Basidiodendron eyrei	AB871753	—	Japan	Sotome et al. 2014
Eichleriella leucophaea	KU726859	*Quercus* sp.	Iran	Ghobad-Nejhad & Langer 2016
Eichleriella leucophaea	KX262136	hardwood	Spain	Malysheva & Spirin 2017
Exidiopsis grisea	AF291281	—	Germany	Weiß & Oberwinkler 2001
Exidiopsis sp.	AF291282	—	Germany	Weiß & Oberwinkler 2001
***Exidiopsis* sp.**	**PQ877708**	**hardwood**	**Hungary**	**This study**
Heteroradulum deglubens	**PQ877706**	***Fraxinus*?**	**Czechia**	**This study**
Heteroradulum deglubens	**PQ877707**	***Corylus avellana***	**Czechia**	**This study**
Heteroradulum deglubens	AF291272	—	Germany	Weiß & Oberwinkler 2001
Heteroradulum deglubens	KX262101	*Sorbus* sp.	Russia	Malysheva & Spirin 2017
Heteroradulum deglubens	KX262112	hardwood	Sweden	Malysheva & Spirin 2017
Heteroradulum deglubens	KX262113	*Corylus avellana*	Estonia	Malysheva & Spirin 2017
Heteroradulum deglubens	MW485791	—	United Kingdom	Ainsworth et al. 2022
Heteroradulum kmetii	KX262122	*Salix nigricans*	Norway	Malysheva & Spirin 2017
Heteroradulum kmetii	KX262124	*Populus tremula*	Slovakia	Malysheva & Spirin 2017

where it is sympatric with *H. deglubens*. Both species are uncommon in the Czech Republic and should be considered for inclusion in the Red List of Fungi (Zíbarová et al. 2024).

Phylogenetic analyses assigned two specimens collected in western Bohemia to *H. deglubens* at more than 99% similarity with other sequences in GenBank (e.g. KX262101, KX262113), whereas its closest relative *H. kmetii* is similar for about 97 % (Fig. 1). The aligned ITS dataset length is 492 characters of which 345 were conserved sites, 135 were variable, 68 were parsimony informative, and 66 were singletons.

The most common habitats of both species in the Czech Republic are often near-natural habitats in protected areas and outside of extensively managed forests. *Heteroradulum kmetii* prefers lowlands, although it has also been found at higher elevation in neighbouring Slovakia from a seemingly rather warm locality. In general, the localities of *H. deglubens* are located at rather higher elevations than *H. kmetii* in the Czech Republic (240–800 m for *H. deglubens*, while 153–440 m for *H. kmetii*). Precipitation at the localities was not found to affect the occurrence of the two species.

Both European species of *Heteroradulum* prefer hardwoods (material examined, Malysheva & Spirin 2017). We observed specimens on dead wood with white rot, either on the ground or attached to dead branches in the canopy.

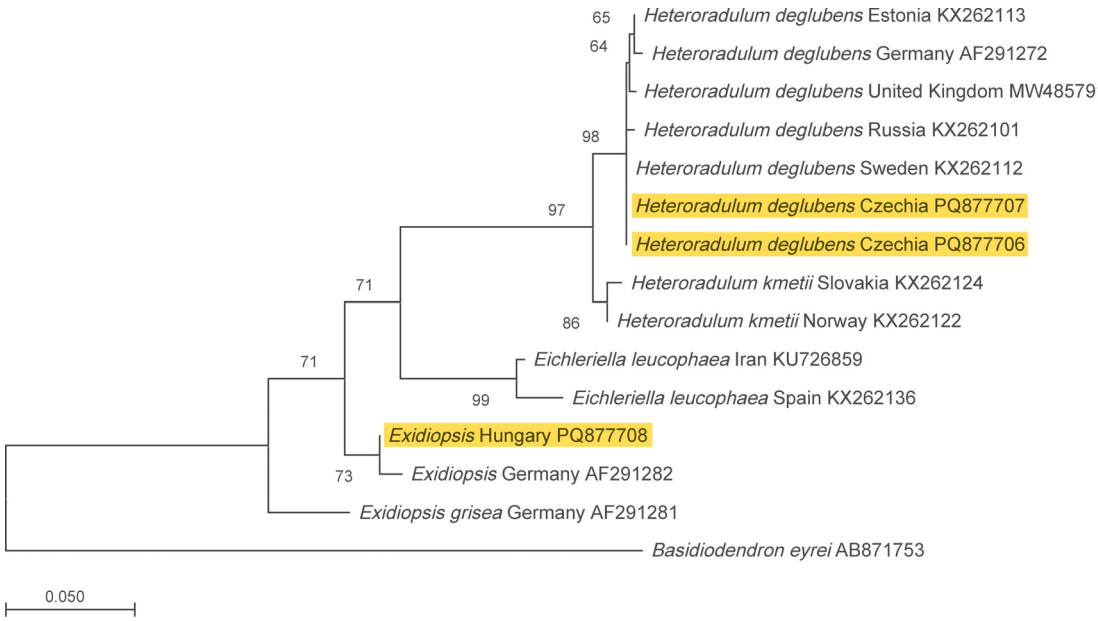

Fig. 1. Phylogenetic tree based on evolutionary analysis of the ITS nrDNA region with the Maximum Likelihood method. Bootstrap support above 50% is shown. Newly generated sequences are indicated in coloured bands.

Heteroradulum deglubens fructifies on decorticated wood of trunks and branches with or without bark and sometimes with mosses. Fruitbodies occur occasionally on woody debris and fresh moss on the wood. Fruitbodies of *H. kmetii* were more often found directly on bark than those of *H. deglubens*. The host substrate of the two species is significantly different. *Heteroradulum deglubens* inhabits a wider range of tree species (most often *Corylus*, *Fraxinus*, occasionally *Sorbus*), whereas *H. kmetii* prefers poplar (mostly *Populus nigra*). This result agrees with Malysheva & Spirin (2017). Moreover, we uncovered additional hosts based on older herbarium specimens: *Crataegus* sp., *Rhamnus cathartica*, and *Cornus sanguinea* for *H. deglubens*, and *Acer campestre* and *Fraxinus angustifolia* for *H. kmetii*.

Heteroradulum deglubens (Berk. & Broome) Spirin & Malysheva, Fungal Biology 121(8): 710, 2017 Fig. 2 a, c, e, g

Basidiomata annual, resupinate, broadly attached; orbicular to elongated irregular in shape, several cm in size; 0.2–1 mm thick. Margin attached, rarely looser, whitish, becoming concolorous with hymenophore. Hymenophore violet pinkish, lighter towards margin, darkening on bruising when fresh; smooth, often

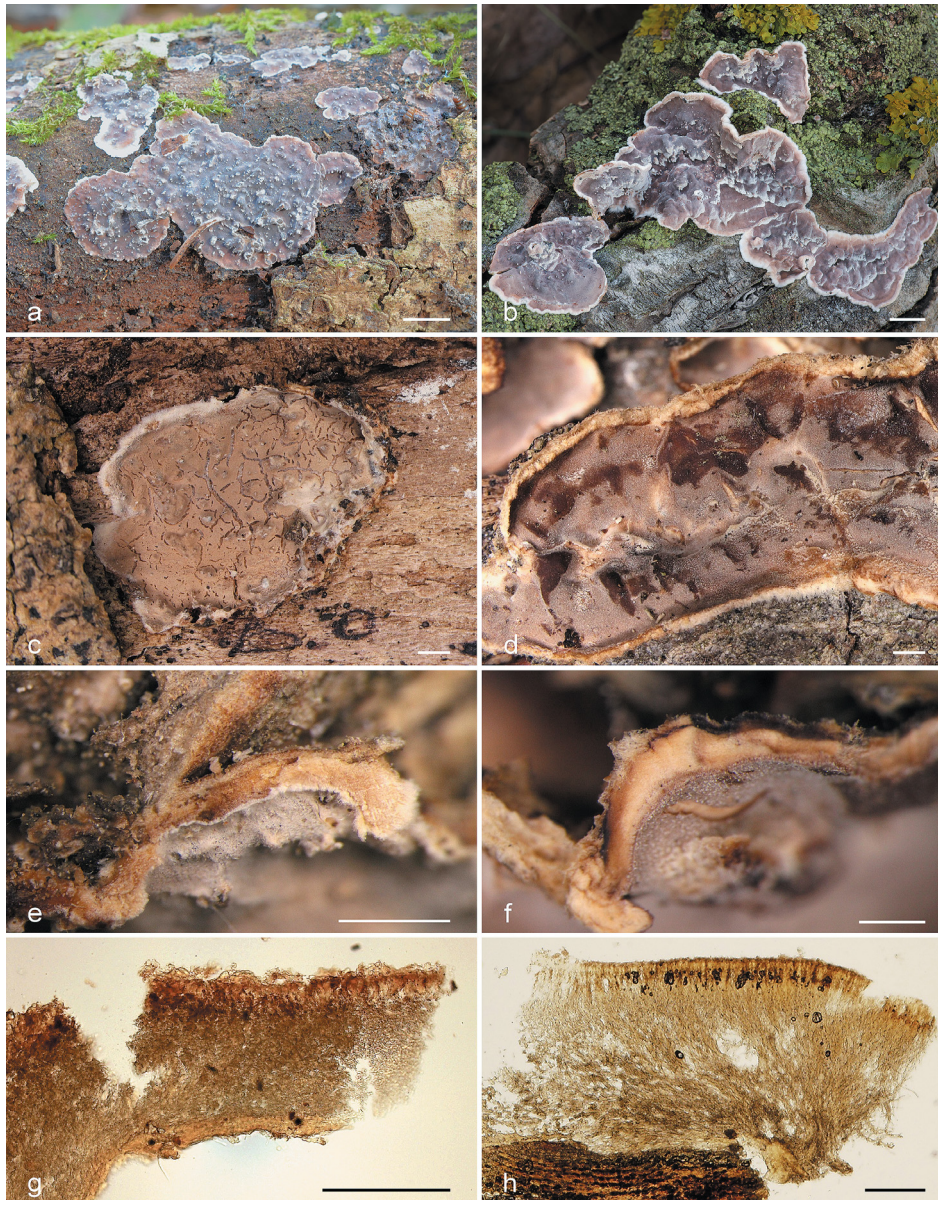

Fig. 2. *Heteroradulum deglubens* (left column): **a** – fruitbodies in situ, Chejlava NNR, Czech Republic (PL BH775); **c** – herbarium specimen, Střela river, Czech Republic (PL BH776); **e** – vertical section through fruitbody (PL BH776); **g** – vertical section through fruitbody under microscope, Černošín, Czech Republic (PL BH777). *Heteroradulum kmetii* (right column): **b** – fruitbodies in situ, Čunovo, Slovakia (HR B002651); **d** – herbarium specimen (HR B002651); **f** – vertical section through fruitbody (HR B002651); **h** – vertical section through fruitbody under microscope, Znojmo, Czech Republic, (BRNM 844562). Scale bars: a–d = 1 cm; e, f = 1 mm; g, h = 200 µm. Photos J. Kout (a, c–h), L. Zíbarová (b).

with irregularly scattered spines, arranged individually or in groups, sometimes forming ridges, simple or richly branched at the top, light-coloured, 0.2–0.5 mm long. Herbarium specimens sordid pinkish, sometimes with ochre tint, with preserved whitish woolly margin, or entire fruitbody unicoloured, hymenophore often irregularly cracked. Some herbarium specimens with naphthalene smell.

Hyphae hyaline with inconspicuous clamps, smooth or incrusted, thin-walled or slightly thick-walled, 2.4–5 µm in diam. Cystidia skittle-shaped. Hyphidia present. Basidia longitudinally septate, club-shaped, 4-spored. Basidiospores cylindrical, slightly curved, smooth, thin-walled, with blunt apiculus, hyaline, with one remarkably large, ellipsoid central drop inside.

Material examined

Czech Republic. Bohemia. West Bohemia. Černošín, border of Pod Volfštejnem NR, on dead lying branch of *Corylus avellana*, covered by mosses, 4 Nov 2023, leg. et det. M. Brzica, J. Kout (KBI, PL BH777; GenBank PQ877707). – Near the village of Obora, Střela river valley, on lying wood (*Fraxinus*?), 22 May 2010, leg. et det. J. Kout (KBI, PL BH776; GenBank PQ877706). – Měcholupy, Chejlava NNR, on lying wood, under *Fraxinus excelsior*, 14 Nov 2018, leg. et det. J. Kout (KBI, PL BH775). – Central Bohemia. [Křivoklátsko], Mt Vosník near the village of Skryje, on lying trunk of *Rhamnus cathartica*, 30 Sep 1979, leg. et det. Z. Pouzar, as *Eichleriella deglubens* (PRM 903445), rev. J. Kout as *H.* cf. *deglubens*. – Žloukovice, near Kabečnice NR, on branch of *Crataegus* sp., 24 Jul 1962, leg. Z. Pouzar, det. W. Wojewoda, as *E. deglubens* (PRM 628154). – South Bohemia. Tábor, Lužnice valley near the village of Řepeč, on dead branch of *Corylus avellana*, 2 Jul 1965, leg. et det. F. Kotlaba & Z. Pouzar, as *E. deglubens* (PRM 756556). – Moravia. Pavlov, Děvín NNR, Mt Obora, fallen trunk of *Rhamnus cathartica*, 23 Oct 2011, leg. et det. D. Dvořák (D.D. 613/11); ibid., on dead branch of *R. cathartica*, 21 Jul 2013, leg. D. Dvořák, M. Kříž, J. Běťák, det. D. Dvořák, as *E. deglubens* (PRM 922445). – [Moravian Karst], Vývěry Punkvy NNR, lying branch of *Fagus sylvatica*, 10 Oct 2008, leg. et det. M. Ainsworth (D.D. 633/08); ibid., lying branch of *C. avellana*, 2 Aug 2022, leg. et det. A. Jirsa (AJ-2022-197). – Valtice, Rendez-vous NNM, on dead branch of *Populus alba*, 14 Oct 1985, leg. et det. F. Kotlaba & Z. Pouzar, as *E. deglubens* (PRM 838036), rev. J. Kout as *H.* cf. *deglubens*. – Bílé Karpaty Mts, Velká Javořina, on lying trunk of *Fraxinus excelsior*, 26 Oct 1972, leg. et det. Z. Pouzar, as *E. deglubens* (PRM 874154).

Poland. Tatry Mts, Mięntusia near the town of Zakopane, on dead trunk of *Sorbus aucuparia*, 10 Nov 1966, leg. F. Kotlaba, det. Z. Pouzar, as *E. deglubens* (PRM 845582).

Sweden. Älvkarleby, Billudden, on fallen trunk of *Sorbus aucuparia*, 20 Oct 1949, leg. et det. S. Lundell (O F-160211).

Switzerland. St. Gallen, Neudorf, valley of Goldach river, on dead branch of *Cornus sanguinea*, 11 Nov 1991, leg. et det. F. Kotlaba, as *E. deglubens* (PRM 873119).

Additional material examined

Hungary. Budapest, Gellért-hegy Jubileumi Park, on attached branch of hardwood, 8 Feb 2024, leg. M. Brzica, det. J. Kout, as *Exidiopsis* sp. (KBI; GenBank PQ877708).

Heteroradulum kmetii (Bres.) Spirin & Malysheva, Fungal Biology 121(8): 711, 2017 Fig. 2 b, d, f, h

The microscopic features of *H. kmetii* are quite similar to those of *H. deglubens*. The most reliable distinguishing features for identification of *H. kmetii* are perennial robust fruitbodies with brownish layers of hymenia separated by tramal

layers in vertical section, effused fruitbodies with uplifted margins which are thick and woolly, sometimes nearly pileate (Tab. 2). For detailed microscopic features of both species, see Malysheva & Spirin (2017).

In addition to the morphological features, the substrate may be useful, and none of the *H. kmetii* specimens had a naphthalene odour. These features are helpful in identifying young resupinate fruitbodies of *H. kmetii*. *Heteroradulum deglubens* and *H. kmetii* can be confused in the field with other stereoid species, mainly with *Stereum rugosum* Pers. However, the microscopic features of the latter are clearly different.

Material examined

Austria. [Lower Austria]. Near Tulln, on bark of *Populus nigra*, Oct, before 1920?, leg. L. & C. Rechinger, det. V. Höhnel, as *Hirneolina Kmetii* (PRM 6702). – [Near Tulln], Langenschönbichl, *Salix*, Jul 1906, leg. O. Bittman, det. O. Bittman?, as *Radulum Kmetii* (PRM 741041).

Czech Republic. Moravia. Podyjí NP, nearby Podmyče, bank of U Jejkala pond, fallen trunk of *Populus tremula*, 24 Aug 2021, leg. et det. D. Dvořák (D.D. 210824-01). – Znojmo, Tvořihrázský les, standing dead *Populus tremula*, 25 Aug 1965, leg. J. Lazebníček, det. J. Lazebníček & H. Kreisel as *Stereum rugosum*, rev. Z. Pouzar, as *Eichleriella deglubens* (BRNM 844562). – Brno-Bosonohy, margin of Bosonožský hájek NR (outside reserve), 49°11'38.1"N, 16°29'48.2"E, dead *Populus tremula*, 5 Sep 2022, leg. et det. D. Dvořák (D.D. 220905-19). – [Former village of] Mušov, lying trunk of *Populus*, 2 Dec 1976, leg. K. Kříž, det. F. Kotlaba (BRNM 306087). – Dolní Věstonice near the town of Mikulov, on dead branch of *Populus nigra*, 30 Aug 1978, leg. M. Tortić, det. Z. Pouzar, as *E. deglubens* (PRM 844582); ibid., on lying branch of *Populus nigra*, 30 Aug 1971, leg. et det. Z. Pouzar, as *E. deglubens* (PRM 871510, PRM 871511); ibid., on lying trunk of hardwood, 30 Aug 1971, leg. et det. Z. Pouzar, as *E. deglubens* (PRM 871524). – Lanžhot, Ranšpurk NNR, on dead branch of *Acer campestre*, 18 May 1966, leg. J. Lazebníček, det. Z. Pouzar, as *E. deglubens* (PRM 871578). – Mikulčice, Skařina forest, on dead branch of *Fraxinus angustifolia* subsp. *danubialis*, 13 Oct 1971, leg. et det. Z. Pouzar, as *E. deglubens* (PRM 870254); ibid., on lying trunk of hardwood, 28 Aug 1971, leg. V. Holubová-Jechová, det. Z. Pouzar as *E. deglubens* (PRM 871516); ibid., on lying trunk of *Populus nigra*, 28 Aug 1971, leg. et det. Z. Pouzar, as *E. deglubens* (PRM 871517), rev. J. Kout, as *H.* cf. *kmetii*. – Strážnice, Muchárov forest, on lying trunk of *P. nigra*, 28 Aug 1971, leg. M. Tortić, det. Z. Pouzar, as *E. deglubens* (PRM 871519). – Litovelské Pomoraví, near Panenský les NR, on *Populus tremula*, 7 Apr 2019, leg. A. Lepšová, det. L. Zíbarová (7042019-1, 7042019-7).

Slovakia. Rusovce, Dunajské ostrovy NR, on fallen trunk of *Populus*, 7 Nov 2019, leg. T. Tejklová & L. Zíbarová, det. L. Zíbarová (HR B002385). – Čunovo, Dunajské luhy Protected Landscape Area, on fallen branch of *Populus*, 5 Nov 2018, leg. T. Tejklová & L. Zíbarová, det. L. Zíbarová (HR B002651). – Prenčov, Havran, on *Populus*, 27 Feb 1898, leg. A. Kmeť, det. A. Kmeť?, as *Radulum Kmeťii*, rev. L. Hagara, as *E. deglubens* (PRM 741040).

Tab. 2. Basic distinguishing features between *Heteroradulum deglubens* and *H. kmetii*.

Feature	***Heteroradulum deglubens***	***Heteroradulum kmetii***
Fruitbody morphology	resupinate	semipileate
Fruitbody persistence	annual	perennial
Hyphal system	monomitic	dimitic
Preferred substrate	*Corylus*, *Fraxinus*	*Populus*

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