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The first record of *Aphanodesmium gabretae* (*Helotiales*, *Leotiomycetes*) on natural substrate reveals a contrasting distribution of its two genotypes

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A short report is given on the first find of the poorly known species *Aphanodesmium gabretae*. This sporodochial anamorphic ascomycete was previously only known as an endophytic isolate from a spruce needle. The newly obtained collection originated from bark of a living spruce twig. Both specimens have an identical morphology, but comparison of their rDNA showed polymorphism. Thanks to the GlobalFungi database, we have been able to demonstrate a contrasting geographical distribution of the two genotypes differing in the rDNA cistron.

Key words: anamorphic ascomycete, *Picea abies*, GlobalFungi, geographical range, phenotype.

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Koukol O., Palice Z. (2025): První nález druhu *Aphanodesmium gabretae* (*Helotiales*, *Leotiomycetes*) na přirozeném substrátu odhalil odlišné rozšíření jeho dvou genotypů. – Czech Mycol. 77(1): 101–108.

V krátké zprávě shrnujeme nález málo známého druhu *Aphanodesmium gabretae*, který byl nalezen vůbec poprvé v přírodě. Tento sporodochiální anamorfní askomycet byl dosud znám pouze jako endofyt z jednoho izolátu ze smrkové jehlice. Nový nález pochází z borky živé smrkové větvičky. Oba vzorky mají identickou morfologii, ale vyznačují se polymorfizmem v rDNA. Díky údajům z databáze GlobalFungi se nám podařilo prokázat odlišné geografické rozšíření obou zjištěných genotypů lišících se v cistronu rDNA.

INTRODUCTION

Aphanodesmium gabretae (Koukol & Kolářová) Réblová & Hern.-Restr. was described based on a single isolate obtained from a surface-sterilised green needle of *Picea abies* in Šumava Mts (Bohemian Forest), Czech Republic. The slowly growing isolate was originally placed in the genus *Bactrodesmium* Cooke because of the morphology of conidia typical of the genus (Koukol & Kolářová 2010). Phylogenetic placement of the isolate at a basal position within the *Helotiales* based on ITS and 28S rDNA sequences was different from the presumed position of *Bactrodesmium* among *Dothideomycetes* (Funk & Shoemaker 1983). Réblová et al. (2020) in their phylogeny confirmed that *B. gabretae* formed a separate lineage in the *Helotiales* and combined the species into the newly established monotypic genus *Aphanodesmium* Réblová & Hern.-Restr.

During a family walk along a stream in Šumava Mts, the second author collected some lichen specimens for routine scientific documentation. One sample from a less usual substrate for lichens – periodically flooded attached twigs (of a young living spruce tree) near the water surface later revealed shiny dark brown to black sporodochia of an anamorphic fungus on the bark. Based on its morphology, the fungus was identified as *Aphanodesmium gabretae*. To our knowledge, this is the first record of this fungal species in the field. The aim of our study was to examine the collection of this rare fungus thoroughly to provide further details about the morphology on natural substrate and occurrence data using the GlobalFungi database.

MATERIAL AND METHODS

The specimen was collected in June 2024, in a mixed forest along a stream, partially formed by a young *Picea abies* plantation and a spontaneously developed alder stand (*Alnus incana*) with interspersed regenerating spruces (Fig. 1 A, B).

After collection, the specimen was air-dried at room temperature. A small part of the sample was used for microscopic study. In the laboratory, individual conidia were picked with a sterile needle and placed on agar plates with malt extract agar (MEA, Oxoid, UK) or potato carrot agar (PCA, HiMedia, India) within five days after collection. Germinating conidia produced sterile white mycelium which was incubated at a temperature of 23–25 °C for several months until sporulation. Sporulating cultures were deposited in the Culture Collection of Fungi (CCF, Prague, Czech Republic), and voucher specimens were deposited in the Herbarium of the Charles University (PRC, Prague, Czech Republic) and Herbarium of the Institute of Botany (PRA, Průhonice, Czech Republic).

To verify the identification using molecular data, DNA was extracted from the mycelium, and two markers were amplified: ITS1-5.8S-ITS2 and 28S regions of the ribosomal RNA gene (rDNA). Further, the gene encoding the second largest subunit of RNA polymerase II (*rpb2*) was amplified using primer set fRPB2-5F/fRPB2-7cR (Liu et al. 1999). Newly obtained sequences were deposited in GenBank.

To extend the presently known occurrence of *A. gabretae*, a search was made in the GlobalFungi database, release 5.0 (Větrovský et al. 2020). Both sequence FN561756 obtained from the ex-type strain and the newly obtained sequence were used. Using the software Seed v. 2.0 (Větrovský et al. 2018), only ITS2 regions were obtained. As they differed in one transition (see below), they were both subjected to an 'Exact hit' search in the database.

RESULTS

Identification of the collection as *Aphanodesmium gabretae* based on its morphology was confirmed by molecular data. The BLAST search performed with the ITS and 28S sequences showed sequences FN561756 and FN561755 (both originating from the ex-type strain of *A. gabretae*), respectively, as the best hits. However, they were not identical, since the ITS1-5.8S-ITS2 sequences differed in three transitions and one deletion, and 28S in two deletions. The newly obtained sequence of *rpb2* showed the most closely related hit for an unpublished collection of *Unguicularia unguiculata* (HG326614) with a similarity of only 89%.

Our searches in the GlobalFungi database substantially differed for both the sequence variants (Tab. 1), suggesting that each of them originates from a genotype with different geographical distribution ranges. The genotype represented by the holotype PRM 915691 was present in 17 samples, while that obtained in the present study (PRC 10533) was present in seven samples, and both variants were present in two samples. For both searches, GlobalFungi attributed the two sequence variants to the same species hypothesis (SH1428465.10FU, '*Bactrodesmium gabretae*').

With one exception (air captured on a montane grassland), all samples originated from temperate forests. All sites but one mentioned *Picea* in the metadata, either as the 'Dominant plant' or 'Other plant'. Geographically, 22 samples originated from Europe (Czech Republic, Austria, France, Germany), while two were from Japan. Eleven samples from Šumava Mts from litter and soil confirmed the occurrence of the species in this region.

Aphanodesmium gabretae (Koukol & Kolářová) Réblová & Hern.-Restr., Studies in Mycology 95: 442, 2020 Fig. 1

≡ *Bactrodesmium gabretae* Koukol & Kolářová, Nova Hedwigia 91(1–2): 244, 2010

Description on substrate. Colonies on natural substrate in the form of sporodochia, circular, mostly single, rarely confluent, pulvinate when young, later flattened, 100–270 µm in diam., superficial or protruding the upper bark layer, composed of a dense mass of pale ochraceous vegetative hyphae covered by dark brown to almost black, shiny conidia, sterile centre exposed when older and conidia produced towards the margin. Conidiophores unbranched, septate,

Tab. 1. Samples from the GlobalFungi database containing one of the genotypes of *Aphanodesmium gabretae* (represented by holotype PRM 915691 and recently collected specimen PRC 10533) and selected metadata as they occur in GlobalFungi. Samples in bold show co-occurrence of both genotypes. Asterisks in Biome details indicate presence of *Picea* as 'Dominant plant' or 'Other plant'. MAP = mean annual precipitation; MAT = mean annual temperature; NA = not available.

Genotype	Sample ID	Elevation (m)	Country	Sample type	Biome details	MAP	MAT	Reference
PRM 915691	GF05019884S	818	Austria	soil	temperate mixed forest	1180	6.2	Bhople et al. (2022)
	GF05019870S	818	Austria	soil	temperate mixed forest	1400	4.5	Bhople et al. (2022)
	GF05019876S	818	Austria	soil	temperate mixed forest	1400	4.5	Bhople et al. (2022)
	GF05019873S	818	Austria	soil	temperate mixed forest	1400	4.5	Bhople et al. (2022)
	GF05027024S	786	Germany	root	temperate forest*	850	6.5	Khokon et al. (2021)
	GF05002060S	1206	Czech Republic	root	temperate mixed forest*	NA	NA	Kohout et al. (2021)
	GF01013716S	1330	Czech Republic	litter	coniferous forest*	1030	5.5	Štursová et al. (2016)
	GF01013719S	1330	Czech Republic	litter	coniferous forest*	1030	5.5	Štursová et al. (2016)
	GF01013721S	**1330**	**Czech Republic**	**litter**	**coniferous forest***	**1030**	**5.5**	**Štursová et al. (2016)**
	GF04004240S	777	Czech Republic	deadwood	temperate mixed broadleaf forest*	1144	5.4	Lepinay et al. (2021)
	GF01010051S	347	Czech Republic	soil	mixed forest*	610	7.5	Mašínová et al. (2016)
	GF04006286S	**1092**	**Czech Republic**	**litter**	**temperate mixed forest***	**1200**	**4.8**	**Odriozola et al. (2021)**
	GF04006265S	1170	Czech Republic	litter	temperate mixed forest*	1211	4.8	Odriozola et al. (2021)
	GF04006279S	1135	Czech Republic	litter	temperate mixed forest*	1192	4.5	Odriozola et al. (2021)
	GF02001056S	96	Japan	soil	temperate mixed forest*	2206	2.8	Ogwu et al. (2019)
	GF02001057S	180	Japan	soil	temperate mixed forest*	2206	2.2	Ogwu et al. (2019)
	GF03008387S	1028	France	air	montane grassland	610	5.3	Tignat-Perrier et al. (2020)
PRC 10533	GF01013720S	1330	Czech Republic	litter	coniferous forest*	1030	5.5	Štursová et al. (2016)
	GF01013721S	**1330**	**Czech Republic**	**litter**	**coniferous forest***	**1030**	**5.5**	**Štursová et al. (2016)**
	GF01013725S	1331	Czech Republic	litter	coniferous forest*	1030	5.5	Štursová et al. (2016)
	GF01013753S	1331	Czech Republic	litter	coniferous forest*	1030	5.5	Štursová et al. (2016)
	GF01013767S	1331	Czech Republic	soil	coniferous forest*	1030	5.5	Štursová et al. (2016)
	GF04004203S	810	Czech Republic	deadwood	temperate mixed broadleaf forest*	1144	5.4	Lepinay et al. (2021)
	GF04006286S	**1092**	**Czech Republic**	**litter**	**temperate mixed forest***	**1200**	**4.8**	**Odriozola et al. (2021)**

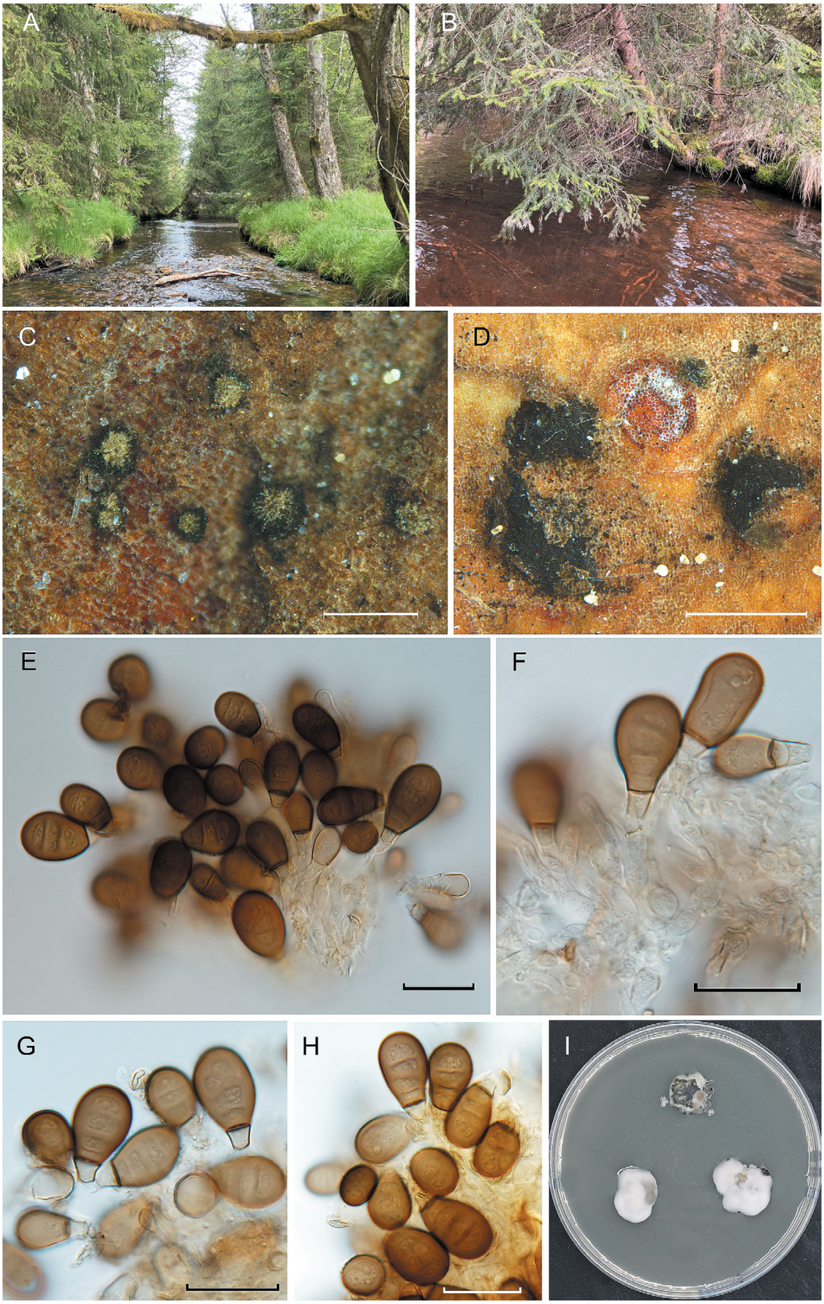

Fig. 1. *Aphanodesmium gabretae* (PRC 10533). **A** – collection site along Hučina stream. **B** – detail of spruce twigs hanging above the water. **C–D** – colonies on bark. **E–H** – conidia. **I** – 6.5-week old colonies on MEA. Bars = 250 µm (C–D), 20 µm (E–H). Photos Z. Palice (A–B), O. Koukol (C–I).

hyaline, (13)17–32(34) × 2.5–3.5 µm, terminated by a single conidiogenous cell. Conidiogenous cells monoblastic, up to 8 µm long. Conidia ellipsoidal to obovoidal or clavate, thick-walled, pale brown with mostly 2–3 transverse distosepta (one of the septa sometimes oblique), 16.5–19.5(22) × 11–14 µm (mean length/width ratio 1.46). Basal cell hyaline, 3–4 × 4–5 µm. Conidia detached rhexolytically.

Description in culture. Colonies on MEA irregular, compact, protruding, white to grey with patches of sporulating regions, reaching 11–15 mm in diam. after 6 weeks.

Specimen examined

Czech Republic. South Bohemian Region, Šumava Mts, Prachatice District, Volary, Černý Kříž, valley of Hučina stream, by bridge near confluence with Lesní potok stream, 48°51'11.3" N, 13°51'47.2" E, 740 m a.s.l., on living twig of young *Picea abies*, 16 June 2024, leg. Z. Palice, det. Z. Palice & O. Koukol, ZP37234 (PRC 10533); ibid., ZP37223 (PRA 22691, ut *Bacidina* sp.). GenBank accessions for PRC 10533: rDNA = PV798863, *rpb2* = PV775782.

DISCUSSION

Aphanodesmium gabretae is mentioned as a species distributed across Europe, but known from only two physical specimens from conifer-dominated forests in the Šumava Mts, Czech Republic. Based on data from the GlobalFungi database, its DNA has also been recorded in litter (Odriozola et al. 2021), soil (Štursová et al. 2016), decomposing wood (Lepinay et al. 2021), and roots (Khokon et al. 2021, Kohout et al. 2021). Most of the samples (14) originate from the Czech Republic, followed by four samples from the Austrian Alps (Bhople et al. 2022). Metadata associated with records in the GlobalFungi database shows occurrence of *A. gabretae* at sites differing in vegetation, elevation and mean annual precipitation. They share a pH lower than 6, mean annual temperature 2–9 °C and most of them also presence of spruce, but its natural or introduced origin cannot be distinguished (Tab. 1).

Numerous records from the GlobalFungi database indicate that *A. gabretae* is more common than expected from only two collections obtained since its description. For such a fungal species with minute colonies, its occurrence may be underestimated by simply being overlooked. On the other hand, it is possible that *A. gabretae* only sporulates on woody parts of spruce, and only at sites directly influenced by the presence of water or in places with long-term air humidity. The twigs with the present collection (Fig. 1B) were hanging above the water level of a stream, which may indicate a requirement for higher humidity.

The newly obtained collection of *A. gabretae* was found sporulating on the surface of bark of a living spruce. Although some sporodochia protruded the bark (Fig. 1D), no other symptoms suggest that this species is pathogenic.

Our search in GenBank confirmed the teleomorphic species *Unguicularia unguiculata* Höhn. as the most closely related taxon (Réblová et al. 2020). However, the low similarities of less than 90% in all studied markers may not be sufficient to consider these two species even congeneric.

No phenotype difference was found when measurements from the present collection were compared with those of the holotype (Koukol & Kolářová 2010). On the natural substrate, *A. gabretae* produced distoseptate conidia almost identical in shape, number of septa, and in size [16.5–19.5(22) × 11–14 µm vs 13–19(24) × 9–13.5 µm]. However, both the ex-type strain and the collection obtained in this study differed in several nucleotides of the rDNA. Presumably, they represent two genetically distinct populations of the same species, an explanation which is supported by our searches with both variants of ITS2 rDNA in GlobalFungi. *Aphanodesmium gabretae* forms two genotypes; one occurring in diverse types of habitats and with a wide geographical distribution across four European countries and Japan, while the other is restricted to mountainous coniferous forests in the Czech Republic (in which they may co-occur). An alternative explanation of the polymorphism in the rDNA cistron is that it is intragenomic. Intragenomic polymorphisms in the ITS region have been documented in members of the *Hypoxylaceae* and various basidiomycetous and ascomycetous yeasts (Stadler et al. 2020). Should the polymorphism at *A. gabretae* be intragenomic, both the ITS2 variants would be present in all samples in the GlobalFungi database.

Our study shows that polymorphism in the ITS region may not only complicate identification of closely related species (Réblová et al. 2020), but may provide further information on the life histories of a given genotype across data in the GlobalFungi database.

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REFERENCES

BHOPLE P., SAMAD A., ŠIŠIC A., ANTONIELLI L., SESSITSCH A., KEIBLINGER K., DJUKIC I., ZEHETNER F., ZECHMEISTER-BOLTENSTERN S., JOERGENSEN R.G., MURUGAN R. (2022): Variations in fungal community structure along elevation gradients in contrasting Austrian Alpine ecosystems. – Applied Soil Ecology 177: 104508. DOI: https://doi.org/10.1016/j.apsoil.2022.104508

FUNK A., SHOEMAKER R. (1983): *Stuartella suttonii* n. sp., the teleomorph of *Bactrodesmium obliquum* var. *suttonii*. – Canadian Journal of Botany 61: 2277–2279.

KHOKON A.M., SCHNEIDER D., DANIEL R., POLLE A. (2021): Soil layers matter: Vertical stratification of root-associated fungal assemblages in temperate forests reveals differences in habitat colonization. – Microorganisms 9: 2131. DOI: https://doi.org/10.3390/microorganisms9102131

KOUKOL O., KOLÁŘOVÁ Z. (2010): *Bactrodesmium gabretae* (anamorphic *Helotiales*), a new sporodochial species described from spruce needles. – Nova Hedwigia 91: 243–248. DOI: https://doi.org/10.1127/0029-5035/2010/0091-0243

KOHOUT P., SUDOVÁ R., BRABCOVÁ V., VOSOLSOBĚ S., BALDRIAN P., ALBRECHTOVÁ J. (2021): Forest microhabitat affects succession of fungal communities on decomposing fine tree roots. – Frontiers in Microbiology 12: 541583. DOI: https://doi.org/10.3389/fmicb.2021.541583

LEPINAY C., JIRÁSKA L., TLÁSKAL V., BRABCOVÁ V., VRŠKA T., BALDRIAN P. (2021): Successional development of fungal communities associated with decomposing deadwood in a natural mixed temperate forest. – Journal of Fungi 7: 412. DOI: https://doi.org/10.3390/jof7060412

LIU Y.J., WHELEN S., HALL B.D. (1999): Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. – Molecular Biology and Evolution 16: 1799–1808. DOI: https://doi.org/10.1093/OXFORDJOURNALS.MOLBEV.A026092

MAŠÍNOVÁ T., BAHNMANN B.D., VĚTROVSKÝ T., TOMŠOVSKÝ M., MERUNKOVÁ K., BALDRIAN P. (2017): Drivers of yeast community composition in the litter and soil of a temperate forest. – FEMS Microbiology Ecology 93: fiw223. DOI: https://doi.org/10.1093/femsec/fiw223

ODRIOZOLA I., NAVRÁTILOVÁ D., TLÁSKALOVÁ P., KLINEROVÁ T., ČERVENKOVÁ Z., KOHOUT P., VĚTROVSKÝ T., ČÍŽKOVÁ P., STARÝ M., BALDRIAN P. (2021): Predictors of soil fungal biomass and community composition in temperate mountainous forests in Central Europe. – Soil Biology and Biochemistry 161: 108366. DOI: https://doi.org/10.1016/j.soilbio.2021.108366

OGWU M.C., TAKAHASHI K., DONG K., SONG H.-K., MOROENYANE I., WALDMAN B., ADAMS J.M. (2019): Fungal elevational Rapoport pattern from a high mountain in Japan. – Scientific Reports 9: 6570. DOI: https://doi.org/10.1038/s41598-019-43025-9

RÉBLOVÁ M., HERNÁNDEZ-RESTREPO M., FOURNIER J., NEKVINDOVÁ J. (2020): New insights into the systematics of *Bactrodesmium* and its allies and introducing new genera, species and morphological patterns in the *Pleurotheciales* and *Savoryellales* (*Sordariomycetes*). – Studies in Mycology 95: 415-466. DOI: https://doi.org/10.1016/j.simyco.2020.02.002

STADLER M., LAMBERT C., WIBBERG D., KALINOWSKI J., COX R.J., KOLAŘÍK M., KUHNERT E. (2020): Intragenomic polymorphisms in the ITS region of high-quality genomes of the *Hypoxylaceae* (*Xylariales*, *Ascomycota*). – Mycological Progress 19: 235–245. DOI: https://doi.org/10.1007/s11557-019-01552-9

ŠTURSOVÁ M., BÁRTA J., ŠANTRŮČKOVÁ H., BALDRIAN P. (2016): Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. – FEMS Microbiology Ecology 92: fiw185. DOI: https://doi.org/10.1093/femsec/fiw185

TIGNAT-PERRIER R., DOMMERGUE A., THOLLOT A., MAGAND O., AMATO P., JOLY M., SELLEGRI K., VOGEL T.M., LAROSE C. (2020): Seasonal shift in airborne microbial communities. – Science of the Total Environment 716: 137129. DOI: https://doi.org/10.1016/j.scitotenv.2020.137129

VĚTROVSKÝ T., BALDRIAN P., MORAIS D. (2018): SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. – Bioinformatics 34: 2292–2294. DOI: https://doi.org/10.1093/bioinformatics/bty071

VĚTROVSKÝ T., MORAIS D., KOHOUT P., LEPINAY C., ALGORA C., AWOKUNLE HOLLÁ S., BAHNMANN B.D., BÍLOHNĚDÁ K., BRABCOVÁ V., D'ALÒ F., HUMAN Z.R., JOMURA M., KOLAŘÍK M., KVASNIČKOVÁ J., LLADÓ S., LÓPEZ-MONDÉJAR R., MARTINOVIĆ T., MAŠÍNOVÁ T., MESZÁROŠOVÁ L., MICHALČÍKOVÁ L., MICHALOVÁ T., MUNDRA S., NAVRÁTILOVÁ D., ODRIOZOLA I., PICHÉ-CHOQUETTE S., ŠTURSOVÁ M., ŠVEC K., TLÁSKAL V., URBANOVÁ M., VLK L., VOŘÍŠKOVÁ J., ŽIFČÁKOVÁ L., BALDRIAN P. (2020): GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. – Scientific Data 7: 228. DOI: https://doi.org/10.1038/s41597-020-0567-7