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Desoria trispinata (MacGillivray, 1896), a promising model Collembola species to study biological invasions in soil communities

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ABSTRACT

The species *Desoria trispinata* (MacGillivray, 1896) is globally distributed but probably not native to Europe. Reports on its occurrence have increased substantially over the last years, proving that it is extremely successful, often dominating in number not only Collembola but the entire macroscopic soil fauna under favourable conditions. Still, *D. trispinata* has seldom been studied, and its systematic position is poorly understood. We compiled available literature on the ecology of this species, rendering information with respect to its success. In addition, during the past years we established a controlled laboratory culture and recorded life history data. Finally, we ran a molecular genetic analysis. COI sequence divergence between specimens of *D. trispinata* was low among specimens from Germany, UK and Russia (up to 0.1%) clearly separating these specimens from other species of the genus *Desoria*. However, distance to specimens of *D. trispinata* from Japan was high (up to 15.3%).

1. Introduction

Collembola are a diverse group of microarthropods (Maaß et al., 2015) that live in most soils and ecosystems (Rusek, 1998). They can occur in high abundances, e.g. up to ~100,000 individuals/m^2 in grasslands (Bardgett and Cook, 1998), and are an essential part of soil ecosystems, e.g. as consumers of fungi and litter (Fountain and Hopkin, 2005). Therefore, and since they are sensitive to soil characteristics and contaminants they are exposed to, Collembola are used in ecotoxicological tests and soil quality assessment (Fountain and Hopkin, 2005; Janus et al., 2015; OECD, 2015).

The role of Collembola in community ecology has frequently been studied, too. To better understand the specific impact of individual species on ecosystem processes, manifold experiments with single species (e.g. Buse et al., 2013; Lartey et al., 1994) or manipulated community composition have been performed (e.g. ÁBear et al., 2012; Cragg and Bardgett, 2001; D'Annibale et al., 2015; Eisenhauer et al., 2011). However, all those studies were restricted to a limited set of clearly defined species that can be cultivated in the laboratory. The same holds true for ecotoxicological tests. Most of them are confined to the standard organism *Folsomia candida*, Willem, 1902, on which the OECD (Organisation for Economic Co-operation and Development) guideline is based (Fountain and Hopkin, 2005). However, there are hints that even different laboratory strains of *F. candida* may differ in sensitivity (Crommentuijn et al., 1995; Diogo et al., 2007). Other species are permitted by the guideline as well if, e.g., these species are unequivocally identified and their reproductive biology is included within the test time, which means their life-history and optimal cultural conditions for growth and reproduction should be known (OECD, 2015).

Further, in recent years, community tests have received increasing attention to take into account sensitivity differences between species (e.g. Filser et al., 2014; Renaud et al., 2017; Scott-Fordsmand et al., 2008). Sometimes specimens for such tests come from various field collections (Buch et al., 2016; Chelinho et al., 2014; Van den Brink et al., 2005), complicating standardisation. The majority of species in culture are euedaphic or hemiedaphic, whereas to our knowledge only very few epigeic species are available. These are mostly *Orchesella cincta* (Linnaeus, 1758; Costa et al., 2012) and *Hypogastrura assimilis* (Krausbauer, 1898; D'Annibale et al., 2015; Scott-Fordsmand et al., 2008). *D. trispinata* is a cosmoplitan species that can occur in high abundances and several habitats (see Sections 3.5.1.1, 3.5.1.2). Back in 1970, Tanaka extensively studied *D. trispinata*, including its culture under laboratory conditions (Tanaka, 1970). However, he cultured the specimens in grassland soil, which is hard to control and difficult to standardize. The only controlled laboratory culture we are aware of had been mentioned in Toft and Wise (1999), who both have retired by now. Thus, their cultures are not available anymore.

D. trispinata is a highly important species as it occurs worldwide and at high abundances (see Sections 3.5.1.1, 3.5.1.2). However, very little

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recent information on the biology of this species is available in Web of Science©. To make the situation even more complicated, its identification is difficult (see Sections 3.1, 3.3). It can be confused with *Isotoma viridis* according to Shaw and Benefer (2015) and it shares characteristics with other Collembola, such as a tridentate mucro with the genera *Parisotoma*, *Pseudisotoma* and *Isotoma* (Fjellberg, 2007; Potapov, 2001). Thus far, its systematic position remains largely unexplored.

To use *D. trispinata* as "standard" test organism it is essential to know whether culturing of the specimens on gypsum-charcoal plates instead of soil is possible, and what are its corresponding life history data. Therefore we attempted to shed more light on the biology of this species. Specifically, we (i) compiled available literature, (ii) scrutinized discrepancies in existing identification keys, (iii) established a laboratory culture under controlled conditions including life history information and (iv) investigated mt COI barcode sequence diversity within our established culture in terms of species identification eligibility.

Our main research questions were:

- Does *D. trispinata* from different locations form a genetically homogeneous cluster that can be clearly separated from other species of the genus?
- Can the species be cultivated for a long period of time on a plaster of Paris/charcoal mixture?
- If so, does it exhibit similar life history data as in cultures with natural soil?
- Are there any recent studies that support the proposed invasiveness of *D. trispinata*?

2. Material and methods

2.1. Literature review

In April 2017 we ran a literature research using Web of Science©, ScienceDirect® and GoogleScholar© with *Desoria trispinata* and its synonyms (see Section 3.1) as keywords.

2.2. Species origin

The specimens were sampled from a population located in Bremen, Germany. In early April 2016 soil cores (depths: 0–4, 4–8 cm, 100 cm^3) were taken within an extensive grassland (53.1301°N 008.8928°E). The dominating ground vegetation is *Holcus mollis*, *H. lanatus* and *Poa pratensis* interspersed with mainly *Plantago lanceolata*, *Rumex acetosa* or *R. acetosella*. In the vicinity of the sampling location several *Betula pubescens*, *Malus* ssp. and a *Carpinus betulus* grow and a few coniferous trees. It is a loamy sand soil with a pH of 3.8 ($CaCl_2$) and a humus content of 4.6% (loss on ignition, 5 h, 650 °C).

Soil mesofauna was extracted using a Berlese-Tullgren funnel apparatus (8 days, 25–60 °C, +5 °C/24 h with 40 °C for 48 h) into transparent, plastic containers (height: 4 cm, diameter: 7 cm) on a 0.5 cm layer of rewetted plaster of Paris with activated charcoal. The aforementioned container height was mandatory since *D. trispinata* tends to jump out of too shallow culture vessels. Collembola were collected daily by hand using a micro-aspirator. Approximately 40 black-coloured Collembola were the initial stock of our culture.

2.3. Preparation and identification

Based on the experiences of Daghighi et al. (2016) we applied two clearing techniques to specimens freshly killed in Ethanol (96%) to identify the technique that cleared the dark pigmentation of *D. trispinata* best and with less tissue damage. Method 1: specimens were transferred to a microscope slide, treated with one to two drops of lactic acid and heated to ~80 °C using a temperature-adjustable heating stirrer (IKA COMBIMAG RCT). Different time steps (30, 45 s and 1, 2, 3, 4 min) were tested. Method 2: specimens were treated with SNET buffer solution in small glass vials and kept at 55 °C for 3 h. Afterwards, the specimens were mounted permanently on microscope slides with Marc André 2 (recipe is given in Dunger and Fiedler, 1997) and assessed under a LEICA MS5 stereomicroscope. The results of clearing were documented photographically (SONY camera U-SMAD) at 100-, 200- and 400-fold magnification. For species determination an Olympus microscope BX60 and keys of Potapov (2001) and Fjellberg (2007) were used.

For a preliminary identification of living specimens via stereomicroscope (see Section 3.1), we distinguished characteristics of living adults based on the experiences with our *D. trispinata*. The characteristics were identified using a LEICA MS5 (magnification 6.3, 16, 25, 40 or an OLYMPUS SZH10, magnification 7, 20, 40). As our specimens laid eggs for the first time within the third week (Table 5), we defined adults as specimens that are older than three weeks. These individuals can be determined by a specific shade of black colour from younger specimens (see Section 3.5.2.7).

2.4. Genetic analysis

Adult specimens were taken from rearing and synchronisation containers, killed and stored in 96% Ethanol until analysis. DNA was extracted using the DNeasy Tissue Kit (Qiagen, Hilden, Germany) with a non-destructive method: following incubation in 110 µl Q-ATL buffer and 3 mAU proteinase K at 56 °C for 36 h, the cuticle was removed, incubated in 30% ethanol to remove remaining buffer salts, and mounted on a slide in Rusek's reagent (Rusek, 1975). The subsequent extraction of DNA followed the manufacturer's standard protocol for animal tissue except for the amounts of extraction reagents, which were reduced according to the reduced amount of tissue lysis buffer initially applied. DNA was dissolved in a total of 40 µl of Q-AE buffer (QiaGen).

The mt Cox1 5′ barcoding region was amplified with the primer pair LCO1490/HCO2198 (Folmer et al., 1994). PCR reactions were performed in 10 µl reaction volumes containing 10–15 ng of DNA, 0.1 µM of each primer (10 µM), 1 x Peq PCR buffer (VWR Darmstadt, Germany), $MgCl_2$ to a total of 1.5 mM, 0.15 mM of each dNTP and 0,25 U of Peq Taq polymerase. DNA amplification was performed under the following cycling conditions: 60 s. of denaturation at 94 °C; followed by 5 cycles with 94°/40 s., 45 °C/40 s., 72 °C/60 s.; 35 cycles as before with annealing temperature increased to 51 °C, and a final extension at 72 °C for 5 min. PCR products were inspected on a 1% agarose gel, single band amplifications were purified with ExoSap-IT (Affymetrix, Santa Clara CA, USA) and sequenced at the BiK-F Bio-diversity and Climate Research Centre, Frankfurt am Main, Germany.

Sequences were manually assembled and checked for ambiguities, deletions and indels as well as adherence to an open reading frame. BLAST searches were performed for all sequences via GenBank online nucleotide database using the megaBLAST algorithm (www.ncbi.nlm.nih.gov, acc. 20.05.2017). Sequences were aligned using Muscle (Edgar, 2004) as implemented in MEGA7 (Kumar et al., 2016). To assess intraspecific variation among sites and samples of available *Desoria* specimens, we estimated uncorrected p distances using MEGA7. Intraspecific p distance was only calculated if more than one specimen per site was available. Newly sequenced DNA sequences were submitted to the BOLD identification system (Ratnasingham and Hebert, 2013), assigned to operational taxonomic units and checked for close match to Collembola sequences present in the BOLD database.

For sequence comparison, we included nine mt COI sequences of *Desoria* species available from Genbank (1 *D. trispinata* each from UK and Russia and 2 from Japan, 2 *D. tigrina* (Nicolet, 1842), 2 *D. olivacea* (Tullberg, 1871), and 2 *D. germanica* (Hüther and Winter, 1961) with accession numbers KT808356 https://www.ncbi.nlm.nih.gov/nuccore/KT808356, JN981079, LC213064–065 https://www.ncbi.nlm.nih.gov/nuccore/LC213064, https://www.ncbi.nlm.nih.gov/nuccore/LC213065, and DQ365787–92 https://www.ncbi.nlm.nih.gov/nuccore/DQ365787,

https://www.ncbi.nlm.nih.gov/nuccore/DQ365788, https://www.ncbi.nlm.nih.gov/nuccore/DQ365789, https://www.ncbi.nlm.nih.gov/nuccore/DQ365790, https://www.ncbi.nlm.nih.gov/nuccore/DQ365791, https://www.ncbi.nlm.nih.gov/nuccore/DQ365792, respectively. All new sequences obtained for this study have been deposited at GenBank® under accession numbers MG001927-1940. Voucher specimens are stored in the GBOL slide collections of Senckenberg Museum of Natural History, DE, Görlitz. Specimen and locality data are accessible online via Edaphobase data warehouse (www.edaphobase.org (Burkhardt et al. 2014)), and in part in the Virtual Microslide Collection (www.virmisco.org (Christian et al., 2014–17)), using either taxon search or collection numbers GR-Col-3027-3040.

2.5. Culturing

Culture conditions predominantly follow the guidelines of the Collembola reproduction test (OECD, 2015) but were adapted: we used transparent, plastic containers (height: 4 cm, diameter: 7 cm). These were closed by their lid but not airtight to prevent anoxia. Their bottom was filled with an approx. 5 mm layer of gypsum with activated carbon (200 g gypsum, 25 g activated carbon, 190 ml demineralised, filtrated tap water). Still, *D. trispinata* can escape from these containers and invade other containers. This occurred more often if they ran out of food, the containers were too dirty or the population density was very high like at the end of the second or within the third month (≥400–600 specimens/container corresponding to 10–16 individuals/cm^2 or 100,000–160,000 individuals/m^2). We prevented escaping and infestation by storing the single culture vessels within a semitransparent and airtight closed plastic box (length: 26 cm, width: 16 cm, height: 11 cm). The culture vessels were exposed to the natural day-night-rhythm in the laboratory and stored at room temperature (20 ± 2 °C).

We watered the containers with demineralised, filtrated tap water 1–2 times a week providing a high relative air humidity but preventing condensation of water droplets. The main food source was granulated dried baker's yeast from the brand Dr. Oetker. Enough food was provided so that it was consumed within three to four days but did not mould. Approximately 2–10 mg were provided twice a week (~1/4–3/4 of a microspatula), depending on the age of the specimens and the population density within the culture containers. Additionally, we offered small (0.5–1 cm^2) twigs or pieces of bark collected from mixed deciduous wood covered by algae and lichens. These were picked up from soil below trees (e.g. *Betula spec.*, *Fagus sylvatica*) and not broken from the trees themselves. Together with some (approx. 5) clay granulate (SERAMIS®), twigs and bark pieces provided structure and food within the artificial habitat; the specimens could hide, climb, graze and lay eggs on them. *D. trispinata* also seemed to thrive better with these structures and additional food source (derived from previous pilot experiments).

To exchange air and keep the balance between providing enough food, e.g. after hatching, but preventing fungal growth, we checked the containers for food, humidity and fungi twice a week. Clay granulates, twigs and bark pieces covered by fungi were replaced and fungi and dead specimens removed. Additionally, clay granulates applied were autoclaved (Varioklav® Steam Sterilizer, type no. 2259) and twigs and bark pieces were frozen at −20 °C before use to avoid contamination with other mesofauna.

Under these conditions the culture of originally 40 specimens increased to several thousand between April and December 2016. To increase the stock culture, start a synchronisation or to exchange specimens from old culture vessels into new ones, we inserted 80–85 specimens (age: > 3weeks) into a new vessel. For age determination see end of Section 3.5.2.7.

For more than 17 months we observed the culture twice a week and frequently re-transferred all specimens from old containers into new ones. We did this by counting all individuals out at the very end of the second month or within the beginning of third month, depending on the population density and number of dead individuals present. This rendered the experience that within the first two months the population usually increased up to 400–600 individuals per container but started declining within the third month. In the third month, or sometimes earlier, oviposition often stopped, too; we did not observe any more new eggs being laid or hatched juveniles. However, after specimens were re-transferred into new containers, oviposition and hatching was resumed. If we omitted this step, the number of dead specimens increased, especially in the second half of the third month. Finally, up to the end of the third or early fourth month, our cultures collapsed: the density decreased below the number of originally inserted adults, sometimes down to zero. Several reasons might drive the collapse:

- The population density was too high (≥400–600 specimens/container corresponding to 10–16 individuals/cm^2 or 100,000–160,000 individuals/m^2). This is more than reported e.g. by Bardgett and Cook (1998) (100,000 individuals/m^2 grassland). High densities may favour cannibalism (Fountain and Hopkin, 2005) or pathogens (Dromph et al., 2001).
- The gypsum-charcoal plates were too dirty, being densely covered with moulted skins. Further, metabolic waste products, fragments or fluids of dead Collembola and microorganisms might have accumulated within the gypsum-charcoal.
- Our specimens reached and exceeded their life span within the third month of a culture vessel (see Section 3.5.3). Together with the stopped oviposition and hatching mentioned earlier, this might contribute to the collapse.

To reduce such collapses and stress of crowding, we transferred specimens via micro-aspirator into new containers, usually by mid to the end of the third month.

2.6. Synchronisation

Most ecotoxicological tests require specimens of synchronized age. To start a synchronisation, adults were inserted into culture vessels as described before but without provision of clay granulate and pieces of twigs or barks to avoid hiding places for eggs and specimens. 20–25 grains of baker's yeast were offered as food. After two days, adults and yeast were removed. As soon as we detected the first hatchlings (day 6, Table 5), we added baker's yeast (10–15 grains).

We started two separate synchronisations in January 2017 (Table 4): synchronisation 1 (start: 11.01.2017) based on adults of one single container. Its parental specimens had been transferred into a new container in October 2016. Synchronisation 2 (start: 25.01.2017) based on a mixture of parent adults from four containers that were transferred last into a new vessel in December 2016. Each synchronisation was run with four replicates and started with 82 adults (similar to stock culture vessels), except for one container (70 specimens in synchronisation 1, not enough specimens left for the last replicate). The four containers of each synchronisation were kept in separate boxes to avoid escaping and entering of specimens between synchronisations. These boxes stood next to each other to ensure comparable storage conditions. We counted the number of F1-specimens (Table 4) by removing them from the containers a few days after they had started laying eggs themselves but before the F2-specimens hatched to record the number of F1-specimens that survived till their first oviposition.

Containers were checked daily with a stereomicroscope, except weekends, to document key events in life history. To allow investigation of colour change by age, we took pictures with a cell phone camera (SAMSUNG GALAXY S III S3 mini, magnification 1) through the stereomicroscope (LEICA MS 5, 20- and 40-fold magnification) and at identical light settings.

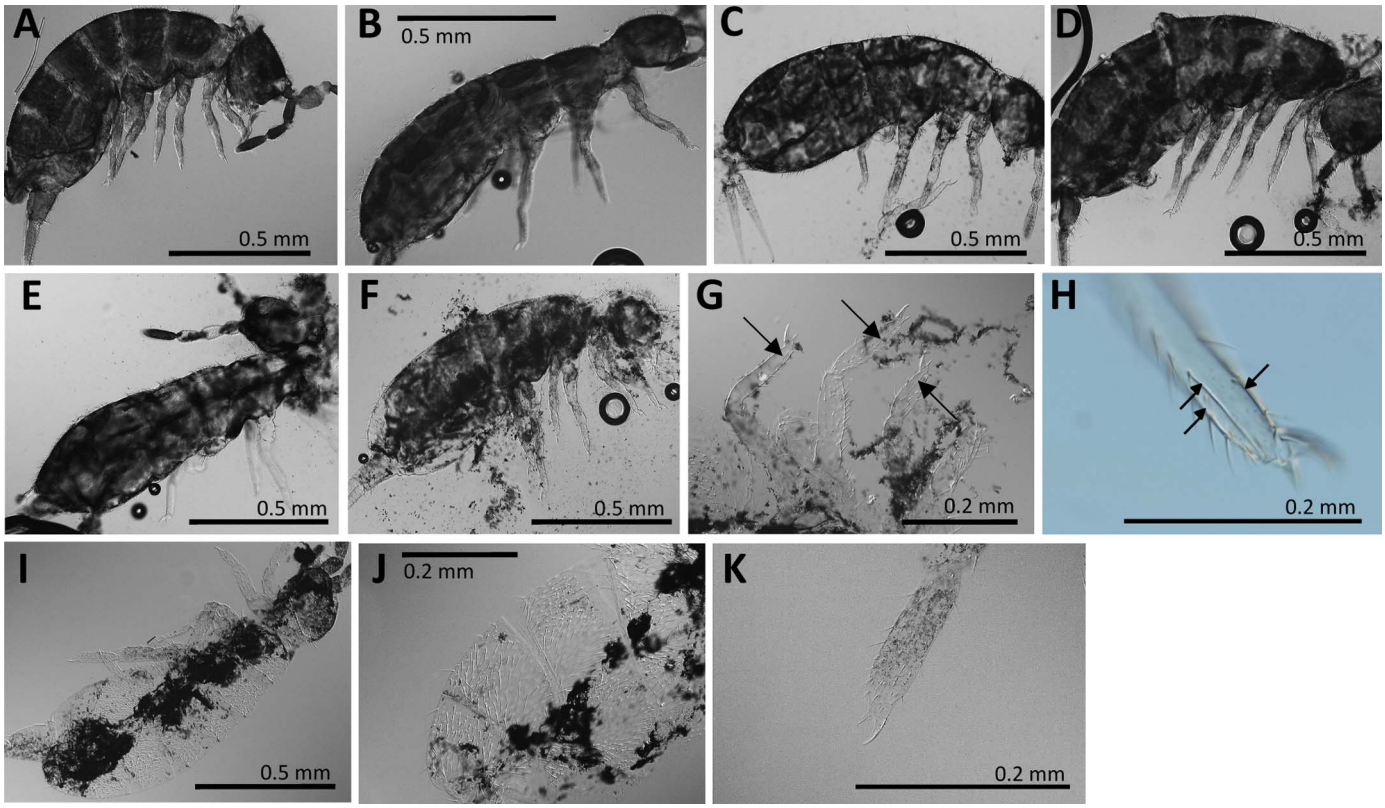

Fig. 1. *Results of clearing specimens of D. trispinata.* Heating (80 °C) in lactic acid for 30, 45, 60, 120, 180 and 240 s cleared the dark pigmentation insufficiently but increasingly caused tissue destruction (A–F). Additionally, artefacts occurred at each tibiotarsus: thin, straight-lined structures that may be distinguished wrongly as clavate tenent hairs (G–H). Specimens treated with SNET buffer solution were cleared stronger with lesser tissue damage (I), the abdominal chaetal pattern was visible (J) and none of the lactid acid artefacts occurred at the tibiotarsus (K).

2.7. Biology and ecology

The information on ecology and biology was compiled using the literature presented in this publication and our experiences with the *D. trispinata* sampled in the field and cultured successfully on plaster of Paris-charcoal for more than 17 months in the laboratory.

3. Results and discussion

3.1. Morphological identification of living specimens

D. trispinata (MacGillivray, 1896) is a little-known Collembola (Shaw and Benefer, 2015) with several synonyms: *Isotoma trispinata*, *Isotoma maritima meridionalis*, *Halisotoma meridionalis*, *Isotoma setinornata* (Potapov, 2001). However, the synonym *Isotoma trispinata* can be mixed up with synonyms of other *Desoria* species (Bellinger et al., 1992–2017): *Isotoma trispinata*, Tuxen, 1944 which is partly synonymized to *Desoria olivacea* (Tullberg, 1871), partly to *Desoria violacea* (Tullberg, 1876). This should be taken into account during literature research.

A preliminary identification of our culture specimens via stereomicroscope showed the following characteristics:

- Slender body shape with a dense cover of short setae that looks like fur
- Body shape Isotoma-like with abdomen widening towards tip and abdomen V and VI separate
- Adults look completely black with whitish basal parts of legs and furca but under a stereomicroscope (full light conditions, magnification ≥20) the black colour sometimes turns into a bluish-purplish black with a vivid, iridescent shimmering (like a drop of oil) (see Fig. 3K).
- D. trispinata is a fast moving Collembola that can jump easily 1–2 cm in distance and > 1 cm in height

The weight of sampled field specimens is on average (dry body mass) 9 ± 1 µg per individual (Van Dooremalen et al., 2013). Newly-hatched individuals are 0.4 mm in length and adults have a mean body length of 0.75 mm whereas a maximum length up to 1.2–1.3 mm (Tanaka, 1970) or even up to 1.5 mm (Bhattacharjee, 1990) is possible.

3.2. Clearing technique

A suitable clearing technique is essential for identification and long-term storage of the prepared specimens. As shown in Fig. 1A–F, lactic acid cleared the specimens insufficiently and increasingly led to tissue destruction. Further, it resulted in loss of some important identification characteristics, especially setae, or artefacts occurred at the tibiotarsi that may be distinguished wrongly as clavate tenent hairs and mislead in identification (Fig. 1G–H). The application of SNET buffer solution cleared the specimens very well, key identifications characterisics stayed intact and were visible very well (Fig. 1I–J) -and none of the lactic acid artefacts occurred at the tibiotarsi (Fig. 1K). Accordingly, we suggest clearing with SNET buffer for *D. trispinata* specimens.

3.3. Identification

For identification after Potapov (2001) and Fjellberg (2007), we mounted specimens cleared in SNET buffer solution in Marc André 2. Important characteristics are: mucro tridentate, apical ring of tibiotarsus with 7–9 setae, tibiotarsi without clavate tenent hairs, but with pointed apical setae, macrosetae short and smooth, especially on abdomen V shorter than tergite length and ventral tube with 3 + 3 laterodistal setae (Fig. 1).

In the identification keys of Potapov (2001) and Fjellberg (2007) many characteristics are identical (e.g. body size up to 1.3 mm) or only slight differences exist (e.g. Ommata with G and H insignificantly smaller versus subequal, PAO 1.0–1.5. times as long as on ommata versus only slightly larger than nearest ocellus). Other characteristics might be not as reliable as they should be for species identification, like the colour of *D. trispinata* (blue or purplish blue versus bluish grey) given the iridescence and colour change by age (see Section 3.5.2, Fig. 3). Further, the number of posterior setae at the dens that is overlapping between keys (14–16 in Potapov (2001), 15–20 in Fjellberg (2007)). However, we detected one relevant contradiction: according to Potapov (2001) the maxillary palp is bifurcate, Fjellberg (2007) stated it is trifurcate. To be aware of such contradictions is essential to avoid misidentification.

Some characteristics of *D. trispinata* can be difficult to distinguish such as the tridentate mucro or the absence of clavate tenent hairs in tibiotarsus II and III (Fig. 1). The latter is a key character separating the genus *Desoria* from *Vertagopus*. According to Potapov (2001) and Fjellberg (2007) no clavate tenent hairs but pointed apical setae should be visible on apical tibiotarsi for precise species determination.

3.4. Genetic analysis

A 662 bp fragment (219 codons) of mt 5′-COI was used for analyses, corresponding to positions 1519 to 2181 of the *Drosophila yakuba* mt sequence (KF824900). No deletions, insertions or stop codons were detected. Among the 24 sequences, there were 434 conserved sites and 224 variable sites. Among the 14 newly sequenced *Desoria* specimens, 30 sites showed a nucleotide ambiguity bias (16 with c/t bias, 8 with a/g, 2 each with a/t, or a/c, and 1 each with t/g, or c/g). Uncorrected CO1 sequence divergence values are shown in Table 1. Genetic distance within *D. trispinata* samples was low, ranging from 0.4 to 1.0% in *D. trispinata* from Germany, Russia and the UK. However, the specimens from Japan were more distant with 13.9 to 15.3% divergence to the other *D. trispinata* specimens investigated. Genetic distance between *D. trispinata* and the other investigated *Desoria* species ranged from 15.5 to 24.0%. A distance phenogram (Fig. S1) placed *Desoria olivacea* and *D. germanica* next to *D. trispinata*, while *D. tigrina* was placed more distant.

Using the specimen identification request tool implemented in BOLD, all our newly sequenced specimens of *D. trispinata* from Germany could be validated via BIN close match to *D. trispinata* as the most similar species available in BOLD, with sequence similarities of our tested individuals to Operational Taxonomic Units present in BOLD ranging between 98.1 and 99.8% (http://www.barcodinglife.org/index.php/IDS_OpenIdEngine, acc. 16.06.2017). One of our sequences was shorter than the minimum 500 bp required for species level barcode identification, however, testing against all barcode records on BOLD still resulted in *D. trispinata* as top hit species. Thus, genetic distance indicated a clear molecular separation between the investigated *Desoria* species, and the low genetic distance between 16 *D. trispinata* specimens from Russia, UK and Germany, compared to the distance to Japanese specimens of this species, so far indicates European *D. trispinata* to represent a genetically homogeneous species regarding its DNA barcoding sequence.

Albeit the number of populations of this species sampled so far for both morphological and genetic identification is admittedly low, their geographical distance and genetic similarity suggest the COI barcoding sequence suitable for molecular based identification and verification of morphological based identification, which, for Palaearctic *Desoria* species, is still a challenging task due the subtlety of morphological characters to be studied, as mentioned above.

Our genetic analyses indicate *D. trispinata* to yield a homogeneous molecular pattern for European specimens that fit the identification definition as presented by Potapov (2001), i.e. a high overall congruence between morphological and molecular species limits as has been revealed for another “Standard” laboratory springtail, *F. candida* (Tully and Potapov, 2015). It has to be kept in mind that *D. trispinata* appears a well-defined species based on its unique morphological character combination, but also outstanding within the genus *Desoria* due to some of these special characters (Potapov, 2001). Presumably being artificially introduced to Europe (Fjellberg, 2007; Potapov, 2001), its genetic homogeneity within this area may be the result of such a comparably fast introduction, as has been proposed for lineage homogeneity of, e.g., *Parisotoma notabilis* (Von Saltzwedel et al., 2017), and further studies, grasping a wider sampling range, may reveal populations with higher genetic divergence, similar to those found in Japan (Potapov et al., 2017). The high genetic divergence of the latter obviously requires further investigation, including investigation of congruence between morphological and molecular based discrimination as proposed by Tully and Potapov (2015), as well as life-history traits and ecological preferences of distinct lineages are required to clarify this issue (Tully and Ferrière, 2008).

Other studies also indicate intraspecific genetic variability of COI being high in Collembola and other soil organisms (e.g. Heethoff et al., 2007; Shaw et al., 2013), and similar high genetic distances between locations but much lower within have been observed in other Collembola species, too, and interpreted as cryptic diversity (e.g. Porco et al., 2012) or high distance between species lineages (Von Saltzwedel et al., 2017) for the same species, here: *Parisotoma notabilis*. For *D. trispinata*, more data are required to resolve these issues.

The aforementioned nucleotide ambiguity found on 30 nucleotide sites within *D. trispinata* was so far only detected among the specimens of our newly studied German population. As this aspect occurred constantly in all three separate sets of amplification runs sent to sequencing, including repeat runs, it cannot be explained by laboratory handling procedures alone or sloppy sequencing, but may be regarded a possible characteristic of this population’s genetic configuration. There are recommendations to remove such ambiguous nucleotide positions as a step of signal cleaning prior to barcoding (Buhay, 2009; Yu et al., 2012). On the other hand, such distinct ambiguous sequence areas may indicate underlying processes of genetic drift, or adaptation to selective pressure, and thus should be held to enable subsequent studies on genetic distinction of soil organisms along the border between species and populations.

3.5. Ecology and biology

3.5.1. Ecology

3.5.1.1. Origin and distribution. *D. trispinata* is probably an invader to Europe originating from the USA (Fjellberg, 2007; Potapov, 2001).

Table 1
Mean sequence distance values (uncorrected p) for *Desoria* species used in the COI analysis, based on 686 bp.

	D. trispinata DE	*D. trispinata* UK	*D. trispinata* RUS	*D. trispinata* JAP	*D. germanica*	*D. olivacea*
D. trispinata DE	0.003					
D. trispinata UK	0.006					
D. trispinata RUS	0.008	0.010				
D. trispinata JAP	0.139	0.146	0.153			
D. germanica	0.167	0.184	0.183	0.174		
D. olivacea	0.192	0.203	0.206	0.188	0.155	
D. tigrina	0.206	0.218	0.222	0.239	0.234	0.240

Table 2
Distribution of *D. trispinata*.

Biogeo-graphic region	Continent	Countries
Holarctics	Europe	Austria (Kindl-Stamatopolos, 2001), Azores (Potapov, 2001), Czech Republic (Potapov, 2001), Denmark and Norway (Fjellberg, 2007), Germany (Fjellberg, 2007; Potapov, 2001; Scholz-Starke et al., 2011; Sticht et al., 2008) and present study, Moldava (Buşmachiu, 2010), Netherlands (Van Dooremalen et al., 2013), Poland (Rzeszowski and Sterzyńska, 2016), Portugal, Italy and Turkey (Özata et al., 2016), United Kingdom (Shaw and Benefer, 2015; Shaw and Ozanne, 2011)
	Asia	Japan (Nakamori and Suzuki, 2007; Potapov, 2001; Tanaka, 1970), Russia (Potapov, 2001; Sharin, 2004), Korea (Lim and Park, 2011; Potapov, 2001)
Palaeo-tropics	Asia	India (Bhattacharjee, 1990; Dorlong, 1984; Hattar et al., 1992; Pahari et al., 2014; Reddy, 1984; Santeshwari and Singh, 2015), Nepal (Potapov, 2001)
	North America	Hawaii (Christiansen and Bellinger, 1992), Mexico (Palacios-Vargas, 2014), USA (Dold, 2010; Giordano et al., 2014; MacGillivray, 1896), Canada (Chagnon et al., 2000; Puvanendran et al., 1997; Skidmore, 1995)
Neo-tropics	South America	Brazil (Andrade et al., 2014; Culik et al., 2002)

MacGillivray (1896) first described it under its synonym *Isotoma trispinata* based on specimens from Salineville, Ohio (MacGillivray, 1896, page 51). But today it is a cosmopolitan mainly distributed in North America and East Asia and with a few records from Europe (Fjellberg, 2007; Kindl-Stamatopolos, 2001; Potapov, 2001). The introduction was likely unintended and occurred e.g. "within soil of ornamental plants, with vegetables, dirty equipment and vehicles" (Zettel, 2010). Publications considering *D. trispinata* increased within the last ten years revealing that it is predominantly inhabiting the Holarctics but also parts of the Neotropics and Palaeotropics, e.g. India, Hawaii or Brazil (Table 2). Habitats are listed in Table 3.

3.5.1.2. Abundance and population density. *D. trispinata* often occurs as the dominant or amongst the dominating Collembola species: in North America *D. trispinata* "is one of the most common species of springtails in surface leaf litter" (Soto-Adames and Taylor, 2010), it was a dominating species in a *Pinus* plantation (Reddy, 1984) and the most dominant species in a pine forest and cultivated land (Hattar et al., 1992). It can even show "the highest density of all species and even among all groups…" (Dorlong, 1984) and be the most abundant Collembola in constructed wetlands (Giordano et al., 2014). Dorlong (1984) recorded densities of *D. trispinata* in India in *Pinus* dominated forest soil and in Jhum soil (an original pine stand now dominated by shrubs and herbaceous vegetation) during the first and second annual cycle. In forest soil, he found densities of 641,000 individuals/m^2 and 686,200 individuals/m^2 respectively, contributing to approx. 83% of Isotomidae, 63% of Collembola and 29% of the forest soil fauna respectively. Densities in Jhum soils were lower: 78.300/m^2 and 91.500^2/m^2 within the first and second annual cycle. Still, they also contributed to > 80% of Isotomidae, > 68% of Collembola and approx. 18% of the soil fauna.

3.5.1.3. Vertical distribution. *D. trispinata* is an epigeic, litter to surface soil dweller: in forest soil approx. 40% were located within litter and approx. 30% at 0–2 cm soil depth whereas in Jhum soil > 75% were located within the first 10 cm (Dorlong, 1984). Tamura (1967) recorded them mainly within the litter and the 0–2 cm soil layer and Tanaka (1970) within the first 5 cm of a grassland soil. In caves, specimens were sampled predominantly (> 99%) from leaf litter (Soto-Adames and Taylor, 2010). This supports that *D. trispinata* is a near surface living species (Van Dooremalen et al., 2013). However, it can occur down to 30 cm soil depth in cultivated land and down to 20 cm depth in pine forests (Hattar et al., 1992).

3.5.1.4. Temperature and humidity. Population densities of *D. trispinata* are mainly affected by soil temperature (Pahari et al., 2014). Tanaka (1970) observed that the duration for hatching, growth until full body length and maturation period became shorter with rising temperature; frequencies of moulting rose with increasing temperature whereas longevity decreased at temperatures higher than 10 °C. In contrast, at 5 °C specimens did not reach maturity. Soto-Adames and Taylor (2010) collected *D. trispinata* in a cave at an air temperature of 15.6 °C, soil temperature 15.5 °C and a relative humidity of 87.3%.

3.5.1.5. Seasonal fluctuations. Tanaka (1970) also studied seasonal effects of precipitation, soil water content, soil and air temperature on *D. trispinata* in Kyushu, Japan (temperature range: 4 °C–30 °C). The vertical distribution was rather unaffected (> 94% of specimens in summer and winter in 0–5 cm soil depth). But size class distribution varied with seasons since the majority of the population in winter (December, February) and in late summer to early autumn (August, September) consisted of adults. Three peaks of population density (February – March, May–June, July) and two minima (April,

Table 3
Habitats of *D. trispinata*.

Group	Type	Habitats
Terrestrial	Forest	**Deciduous**: oak pine (Toft and Wise, 1999), sugar maple (Chagnon et al., 2000), oak, redwood (Özata et al., 2016), **Coniferous**: protected forest (forest site: dominated by Pinus kesiya; Jhum site: original pine stand now dominated by shrubs and herbaceous vegetation) (Dorlong, 1984), Pinus plantation (Reddy, 1984), Pine (Hattar et al., 1992), commercial forest of Abies balsamea (Puvanendran et al., 1997)
	Grassland	MacGillivray (1896), Tanaka (1970), Christiansen and Bellinger (1992), Sticht et al. (2008), Scholz-Starke et al. (2011), van Dooremalen et al. (2013), present study
	Cultivated land/ agriculture	Tamura (1967), Hattar et al. (1992), Dold (2010)
Water-associated	Wetlands	Constructed wetlands (Giordano et al., 2014)
	Rivers	Within wet sediment of an inner-city riverbank (Kindl-Stamatopolos, 2001), Mangrove (Andrade et al., 2014)
	Caves	Occasionally in caves (Kindl-Stamatopolos, 2001), caves (almost exclusively from leaf litter) (Soto-Adames and Taylor, 2010)
Anthropogenic	Urban	Aboveground biotops (Kindl-Stamatopolos, 2001), indoor flower pots, outdoor house walls, in compost and other organic debris (Fjellberg, 2007), oak leaf mulch from compost heap (Shaw and Ozanne, 2011), urban green (Rzeszowski and Sterzyńska, 2016)
Others	Volcano and high altitudes	Wetland at the top of a volcano (Lim and Park, 2011), mofette fields (in plots with 100% soil CO_2 concentration) (Russell et al., 2011), different altitudes within the Darjeeling Himalaya (Pahari et al., 2014)

September) were detected. This pattern partly coincides with the results of Dorlong (1984) (site in Meghalaya, India; temperature range: 2–26 °C): he described maxima in litter and 0–10 cm soil depth for June and July and a minimum in autumn (September, October, November) but also a minimum, not a maximum, in winter months (December, February).

3.5.2. Biology

3.5.2.1. Sex. Males and females have been observed by Bhattacharjee (1990) who investigated amongst others the germcell cytology. Fjellberg (2007) did not observe males within *D. trispinata* from Fennoscandia and Denmark. According to Tanaka (1970) it is impossible to distinguish between sexes of living individuals.

In old culture containers (third month after re-transferring of specimens) we observed differences in size and shape between fully grown adults. Some were slightly bigger and the abdomen was more roundish, like those of e.g. *Protaphorura*. Other specimens were slightly smaller with an overall cane like shape and without a roundish abdomen. We suspect the bigger roundish ones to be females, maybe before oviposition. We did not observe any behavioural differences between the suspected males and females while we took care of the culture, e.g. regarding reproduction. However, a) to distinguish between sexes was not a focus of our research and b) the aforementioned differentiation in suspected living males and females needs some experience with this species changing by ageing to be detected and it is prone to error. Consequently, we concur with Tanaka (1970): it is impossible to reliably distinguish sexes in living individuals.

3.5.2.2. Eggs and hatching. The whitish to amber-coloured eggs were often laid in clutches but also singly as known from other Collembola (Butcher et al., 1971) and *D. trispinata* (Tanaka, 1970). *D. trispinata* oviposited nearly everywhere but preferred the offered clay granulates, the vicinity of twigs and bark pieces, holes within the charcoal gypsum layer or the edge of the containers. Shortly before hatching the pigmented ommata became visible within the semitransparent eggs (Fig. 3A) as described e.g. for *Orchesella cincta* (Schaller, 1970). Juveniles usually hatched head first (Fig. 3B) but occasionally we observed hatching abdomen first.

3.5.2.3. Feeding behaviour. D. trispinata is a detritivorous species (Zettel, 2010) that may be attracted by fungal volatiles (Giordano et al., 2014); e.g., dead specimens were found on fruit bodies of basidiomycetes (*Russula bella*) (Nakamori and Suzuki, 2007).

To the first 40 specimens of our culture we offered different food sources: oat flakes, granulated, dried, baker's yeast as well as twigs and bark pieces of deciduous wood collected from the study site. First, *D. trispinata*, probably starved after the living extraction, ate everything. After two days they favoured baker's yeast and grazed on algae growing on twigs and bark pieces. However, they never completely grazed all algae but did so with the granulated baker's yeast. Thus, we chose these two as food sources for the culture.

3.5.2.4. Cannibalism. At two occasions we observed potential cannibalism: *D. trispinata* fed on dead specimens or eggs. One time an adult fed on a juvenile that had died recently during hatching. For about two minutes the adult gnawed on an area between egg shell and juvenile and at the juvenile. On another occasion, an approximately one week old juvenile fed on the neck of a dead younger juvenile (approx. 2–3 days old). The body of the dead specimen shrunk during that activity.

At each of these occasions food was consumed completely and the population density was very high. ≥400–600 individuals lived within the container corresponding to 10–16 individuals/cm^2 or 100,000–160,000 individuals/m^2 respectively. This reached and exceeded the range of field densities (e.g. > 100,000 Collembola/m^2 grassland, (Bardgett and Cook, 1998)) and according to Fountain and Hopkin (2005) densities of more than one *F. candida*/cm^2 reduces egg deposition and can increase cannibalism of eggs. In addition, it is known that lack of food may cause *F. candida* to cannibalize its own eggs (OECD, 2015). To avoid cannibalism of *D. trispinata*, too high densities and lack of food should be avoided. This is another reason why we fed our culture twice a week and transferred specimens to new containers at the latest by the end of the third month.

3.5.2.5. Movement. Under the stereomicroscope conditions (intense light, open lid supporting air exchange) *D. trispinata* moved fast on the plaster of Paris-charcoal layer of our culture containers. They can jump > 1 cm in height and 1–2 cm in distance and climb up easily the walls and at the lid of vessels; a behaviour also mentioned by Tanaka (1970) for specimens reared in culture tubes with soil.

3.5.2.6. Moulting. D. trispinata is moulting during its entire life. At no occasion we saw them eating the moulted skin, and within three months the culture vessels were covered with a layer of white exuviae. Thus, it can be reasonably assumed that *D. trispinata* is not eating it. This is an important way to get rid of waste products or pollutants potentially stored within or attached to the moulted skin, which, in Collembola also contains the midgut epithelium (Filser and Hölscher, 1997; Vijver et al., 2004). After transferring specimens into new vessels they changed skin within the first three days, often before laying the first eggs. Pigments were likely recovered before ecdysis: a few days before moulting adults turn greyish black and the exuviae stripped were white whereas dead specimens are black (Fig. 2). Instead, adults after ecdysis were shiny black for a while.

3.5.2.7. Colour change by age (Fig. 3). New hatchlings (≤24 h) looked nearly transparent, like whitish glass without any pigmentation except from the black ommata. Juveniles turned into a partly dirty-greyish or

Fig. 2. (A) Difference in colour between a freshly moulted (shiny black) *D. trispinata* and a specimen that will change its exoskeleton soon (greyish black). (B) An adult stripping the white remains of an exoskeleton. (C) A dead adult coloured in black with whitish tips of furca and legs. In the upper right corner a whitish exuviae is visible.

bluish anthracite mixed with light pink (day 2–3) stepwise darkening into a purple with slightly bluish shades (day 7). By the end of week three up to the middle of week four, the colour had changed slowly into a dark midnight bluish violet. At daylight conditions, this colour looked already like the black of the fully grown adults, but it was not. When assessing these specimens with a stereomicroscope at higher magnifications (20–fold) and under full light conditions they looked very dark blue to violet. However, within the fourth week, the first specimens turned into the final black of the adults, even under the aforementioned stereomicroscope settings. By end of the sixth week, all specimens had turned into the black of the adults.

The early steps in colour change by age of our *D. trispinata* partly resemble those described for *Hypogastrura purpurescens* (Lubbock, 1867) by Strebel (1932). In contrast, Tanaka (1970) described his Japanese juveniles as gradually changing from clear white to light blue. However, specimens of *D. trispinata* exhibit a broad variety of black, blue, green or purple (Bellinger et al., 1992–2017). This change in colour by age of our specimens enabled us to distinguish four age stages by stereomicroscopial inspection (magnification 20-fold, full light conditions):

- Fresh hatchlings (≤24 h): nearly transparent, like whitish glass without any pigmentation except from the black ommata.
- Juveniles (2–3 days): semitransparent, a mix of dirty-greyish or bluish anthracite mixed with light pink.
- Juveniles (~7 days): completely coloured, purple with slightly bluish shades.
- Adults (≥4–6 weeks): completely pitch black, even under the stereomicroscope conditions mentioned above.

3.5.3. Life history of gypsum-charcoal cultured populations

Some life history data were assessed by us and Tanaka (1970). However, differences in culture conditions exist between both of us we shortly want to summarize since they might cause differences in life history. He reared cultures on a 0.5 cm layer of field soil (sieved, mesh size 0.25 mm) in petri dishes (9 cm diameter) providing dry yeast once a week and keeping them at room temperature. Specimens were kept in glass tubes (13 × 55 mm) at 20% soil water content and at different temperatures, food was offered constantly and glass tubes were checked every two to three days (5–20 °C) or daily (25 °C, 30 °C). In contrast, we cultured *D. trispinata* in plastic containers (7 cm diameter, 4 cm height) on plaster of Paris-charcoal as recommended for ecotoxicological test cultures (e.g. OECD, 2015). Stock culture containers were checked twice a week and synchronisation containers daily, except weekends, to offer moist conditions and yeast constantly. All vessels were stored at ~20 °C room temperature. The main design and results of the synchronisations our life history data based upon are summarized in Tables 4 and 5.

Our egg development time (6 or 8 days, Table 5) resembled those of Tanaka (1970) (4.8 or 9.5 days, cultured at 20 °C and 25 °C). The number of F1-individuals differed between our two synchronisations (Table 4); however, both were not significantly different to each other (Wilcoxon rank sum test, p-value = 0.6857). A few F1-generation specimens died a few days after they had started laying eggs. During the daily observations these were removed from the containers. However, their percentage was low: in total, ≤3 specimens per container died, corresponding to < 5% (Table 4). Thus, the high variation in F1-specimens might be rather due to different male/female ratios inserted into the single replicates because of the aforementioned impossibility to distinguish sexes in living specimens, or due to age differences of specimens inserted. This is relevant since fecundity rate is age-specific in *D. trispinata* (recently matured females laid eggs less often than the average) and follows oscillating patterns (Tanaka, 1970).

Table 4
Characteristics of synchronisation 1 and 2 with *D. trispinata* (four replicates).

	Synchronisation 1				Synchronisation 2			
Culture from	10/2016				12/2016			
Start Synchronisation	11.01.2017				25.01.2017			
Adults inserted	82	82	82	70	82	82	82	82
Living F1 specimens	79	158	172	47	39	161	98	51
Dead F1 specimens	0	2	0	1	1	0	3	2

At maturity (time of first oviposition), the mean age of specimens was 17 or 18 days (Table 5) and their mean size approximately 0.7–0.8 mm (Fig. 3). The specimens kept growing after the third week as described by Tanaka (1970). We did not measure body length like Tanaka did; but based on the experiences with our stock culture and Fig. 3E our specimens likely grow up to a maximum length of approximately 1.3–1.5 mm. This corresponds to other references (Bhattacharjee, 1990; Fjellberg, 2007; Potapov, 2001; Tanaka, 1970).

Tanaka (1970) recorded a decrease in mean longevity at birth with increasing temperature above 10 °C (54 days at 20 °C, 33 days at 25 °C). We did not assess the mean life span. However, the life history data of the synchronisations and the timing of the population collapse of our stock cultures gives some indication on the maximum life span of our specimens. (i) Within 24 h after re-transferring specimens from old to new culture vessels eggs were laid. Therefore, the parental individuals should be at least 17 days old according to the synchronisation data (first oviposition after 17–18 days). (ii) The populations in culture vessels usually collapsed in the third month, after 8–12 weeks (see Section 2.5). This together adds up to an age of 73–101 days of the parental specimens inserted. The F1-specimens were only 6–8 days and the F2–specimens ~ 30 days younger than the original parental specimens (Table 5). The survivorship curves after hatching (20 °C) of Tanaka (1970) displays that ~75% of the specimens did not survive longer than 75 days and that only ~10% survive longer than 100 days. This corresponds with the age of our specimens at the mass dying within the third month. Thus, our specimens likely have a similar maximum life span than reported by Tanaka (1970) although this is a calculated maximum life span based on combining findings of mixed cultures (stock culture and synchronisation).

Summarizing it can be said, that egg development time, size and maximum life span of our *Desoria* specimens cultured on gypsum-charcoal were similar to *Desoria* specimens cultured in natural soil (Tanaka, 1970). Further, some life history data also resemble those of *F. candida* which eases handling for researchers already familiar with *F. candida*. At 20 °C *D. trispinata* laid eggs within 24 h, the F1-generation hatched at day 6 or 8 and laid eggs themselves at day 17 or 18. *F. candida* F1-specimens hatched within 7–10 days and laid eggs after 3 weeks (Fountain and Hopkin, 2005; Stam et al., 1996). The handling for rearing and synchronisation (see Sections 2.5 and 2.6) is also similar to those of *F. candida* which makes it a promising candidate for ecological and ecotoxicological studies.

3.5.4. How invasive is Desoria trispinata?

As we have shown in Section 3.5.1, *D. trispinata* is globally distributed. When tracking the records of the species over time, MacGillivray's description (1896) refers to Salineville, Ohio, US. After that, the species was recorded in Japan (1970), India (1990) and Hawaii (1992), followed by Canada (1995), Southern and Central Europe, Korea, Nepal and Russia (2001), Brazil (2002), Scandinavia (2007), Moldavia (2010), Great Britain (2011), The Netherlands (2013), Mexico (2014), Poland and Turkey (2016); (references see Table 2). Although this is not a precise chronosequence and we cannot distinguish if the pattern was caused by research activity or actual occurrence it is conceivable that *D. trispinata* originates from Asia and was afterwards transported to other countries via potting soil, as suggested by Fjellberg (2007) and others. We also demonstrated that the species occurs at high to very high population densities (up to almost 700,000 individuals/m^2), often dominating Collembola, sometimes even the entire microarthropod

Table 5
Life history of *D. trispinata* investigated by two synchronisations.

	Days of synchronisation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Synchronisation 1	Adults inserted	x	x																												
	Adults inserted laid first eggs	x																													
	First hatching of F1								x																						
	≥90% eggs hatched										x																				
	F1 laid first eggs																								x						
	First hatching of F2																													x	
	Days of F1 – generation								1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Synchronisation 2	Adults inserted	x	x																												
	Adults inserted laid first eggs	x																													
	First hatching of F1						x																								
	≥90% of eggs hatched									x																					
	F1 laid first eggs																							x							
	First hatching of F2																												x		
	Days of F1 – generation						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

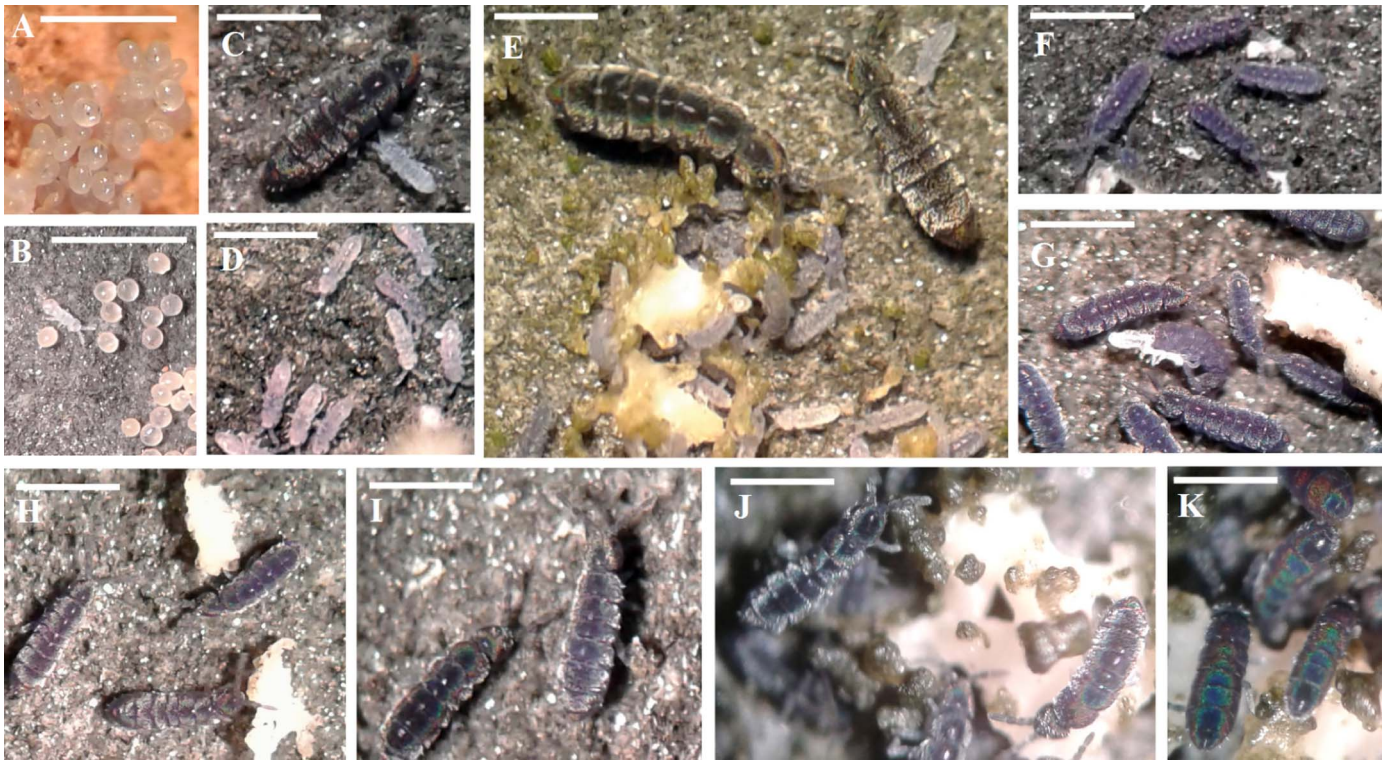

Fig. 3. Development of *D. trispinata* and its change in colour by age. (A) Eggs with black pigmented ommata. (B) Hatchling leaving an egg shell. (C) Juvenile (≤24 h) together with an adult (25 days). (D) Juveniles at day 2. (E) Fully grown adults (≥4–6 weeks) with juveniles of different ages (≤7 days). Specimens at day 7 (F), 14 (G), 21 (H), 25 (I) and 37 (J). (I–J) Specimens changing from dark midnight bluish-violet into the pitch black of fully grown adults (fourth to sixth week). (K) Colourful iridescence pattern reflecting on the dense cover of short setae. These setae are visible under the microscope very well (Fig. 1J). Scale bars 0.5 mm.

community.

But why is it so successful? First of all, definitely due to its very short time to reproduction, large clutch size and a high percentage of successfully hatching juveniles (see Section 3.5.3. and Table 4). Apparently there aren't also any specific habitat requirements. Habitats can be summarized in four main groups. *D. trispinata* occurs in terrestrial (e.g. in forests, grasslands, cultivated lands), water-associated (e.g. wetlands, river banks, caves), anthropogenic areas (e.g. towns, house walls, flower pots) or in other locations (e.g. associated to volcanic regions) (Table 3).

Moreover, also the tolerance limits of the species appear to be very wide, both with respect to several abiotic factors and management. For instance, our regional population thrives at a pH as low as 3.8 (Section 2.2). *D. trispinata* can cope with the extremely high CO_2-concentration at mofette fields (Russell et al., 2011, see Table 5); and it has a high resistance to gamma radiation, which is higher than for other Collembola (Loring, 1985). The species "hardly reacted to clear cutting or shifting agriculture" (Dorlong, 1984). This is perhaps compensated by a shift in vertical distribution. Hattar et al. (1992) compared cultivated versus forest land: in 0–10 cm soil depth densities were twice as high in a cultivated lawn as in a pine forest "and reverse in the case of 10–20 cm soil layer."

Finally, another reason related to biotic interactions could contribute to the success of *D. trispinata* - it is a toxic prey for some predators: Wolf spiderlings (*Schizocosa*) that fed exclusively on *D. trispinata* did not growth, died without moulting, and hatchlings feeding on them survived less than those of the starved controls (Toft and Wise, 1999).

4. Conclusions

D. trispinata was originally described from Salineville, Ohio (MacGillivray, 1896) and later on intensively studied in Japan by Tanaka (1970). Today it is spread over India, Korea, Hawaii, the US, Canada, Central and South America, Europe, Russia and Turkey. The sequence of records seems to indicate that western areas of the Palaearctic are the territory of invasion of this species, which is possibly originated from either the East Palaearctic or the Nearctic.

We successfully established a controlled laboratory culture of *D. trispinata* for more than 17 months by now, verified its taxonomic position and recorded life history data. The species reproduces very fast (from ~85 to 400–600 individuals within 3 months). Analysing the available literature showed that it is a very successful species, as may be explained by a short life cycle, resistance against various adverse abiotic conditions, and toxicity to lycosid spiders - abundant predators controlling other Collembola populations. The combined information gives strong support for a highly invasive potential of the species.

A genetic analysis assembled specimens of our culture and *D. trispinata* from the UK and Russia in the same cluster, which was clearly separated from other species of the genus. To complete the knowledge on *D. trispinata* and its systematic position we need more verified records and genetic analyses. The use of different identification keys and rechecking the characters listed in the genus and species description of the keys is mandatory. Inviting experienced experts in scrutinizing for a final determination of the species is another option. Finally, a genetic community analysis consisting of DNA barcoding can assist species identification and reveal further information.

This cosmopolitan species should be considered more often for ecological and ecotoxicological studies. It can easily sampled in the field, extracted alive and cultured in the laboratory; moreover we offer to share specimens of our well-characterized culture.

This would prevent that not enough specimens exist for a test as in Van Dooremalen et al. (2013) and increase ecological understanding of an introduced widespread, dominant and probably invasive Collembola species.

Although published evidence on *D. trispinata* is increasing many questions still remain open - in particular to find out the main reasons for the species' invasiveness. Based on our findings, priority should be given to (i) more details on populations, such as sex ratio, possible parthenogenesis, clutch size or frequency of oviposition (effects on growth rate); (ii) additional field studies with particular emphasis on origin and phylogenetic position (how similar are distant populations?), community composition and predators (can we identify any successful antagonists or mutualists?) and long-term development (does the dominance increase or stabilise over time?); (iii) laboratory experiments with other predators and competitors (details on mechanisms of interactions, e.g. detoxification, niche separation); and (iv) ecotoxicological studies with respect to sensitivity of *D. trispinata* compared to other species (is *D. trispinata* more resistant towards pesticides than other species?).

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