Cryptic diversity within grass-associated *Abacarus* species complex (Acariformes: Eriophyidae), with the description of a new species, *Abacarus plumiger* n. sp.

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Abstract

Accurate estimation of species richness is often complex as genetic divergence is not always accompanied by appreciable morphological differentiation. In consequence, cryptic lineages or species evolve. Cryptic speciation is common especially in taxa characterized by small and simplified bodies, what makes their proper identification challenging. The cereal rust mite, *Abacarus hystrix*, was regarded for a long time as a species associated with a wide range of grass hosts, whereas wide host ranges are rather rare in eriophyoid mites. Therefore, the generalist status of *A. hystrix* was questioned. In this paper we demonstrate that the diversity within *Abacarus* species associated with grasses is more complex than it was previously thought. The 78 *Abacarus* mtDNA COI sequences used in this study formed 10 highly supported clades (bootstrap value 99%) and four more distinct genetic lineages were represented by unique sequences. The genetic distances between them ranged from 6.6 to 26.5%. Moreover, morphological study and genetic approach based on the combination of the Poisson Tree Processes model for species delimitation (PTP) and a Bayesian implementation of PTP (bPTP), and Neighbour Joining analyses led to delimitation of a new species within the *Abacarus* complex: *Abacarus plumiger*, specialized on smooth brome (*Bromus inermis*). Furthermore, our analyses demonstrated a pattern of host-associated differentiation within the complex. Overall, our study indicates that cryptic speciation occurs in the grass-associated *Abacarus* genus, and suggests the need for more extensive sampling using integrative methods.

Keywords Barcoding · Biodiversity · Cryptic species · Eriophyoid mites · mtDNA COI · Taxonomy

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Introduction

The genetic divergence leading to speciation is not always accompanied by appreciable morphological differentiation. In consequence, cryptic lineages or species evolve and their occurrence, being often overlooked, contributes to the underestimation of species richness (Gerhardt 2005; Stuart et al. 2006; Bickford et al. 2007; Pfenninger and Schwenk 2007; Ceballos and Ehrlich 2009; Wan et al. 2014) and although cryptic species are widespread and common, the magnitude of cryptic diversity remains unknown (Bickford et al. 2007; Pfenninger and Schwenk 2007; Stork 2018). Recognition of the extent of hidden diversity within any taxonomic group is essential to assess its overall diversity and to understand the complexity of its ecological interactions and evolutionary histories (Bickford et al. 2007; Skoracka et al. 2015). Furthermore, evaluation of cryptic diversity is of special importance for conservation biology, as well as for research on parasites and agricultural pests (Liu et al. 2012; Poulin 2014).

Over the last 30 years, there has been an exponential increase in the number of studies that demonstrated the existence of cryptic diversity (Bickford et al. 2007; Poulin 2014; Espíndola et al. 2016; Živković et al. 2017). This rising trend is linked to increasing access to wide array of technologies that enable effective species delimitation. For example, employing super-resolution microscopy, such as electron or confocal microscopy, resulted in the deeper examination of morphology and ultrastructure details that could not be done with classical light microscopy (Chetverikov et al. 2013). Furthermore, increased access to molecular biology techniques had led to the development of barcoding procedures, which allowed fast species delimitation and discrimination on the genetic basis. As a result, many species that were initially described with traditional morphology-based methods as single taxonomic units, were later revealed to be complexes of cryptic species (Knowlton 1993; Funk et al. 2012; Skoracka et al. 2015). However, many of them, before correct identification with aid of genetic methods, had been suspected to be species complexes on the basis of their biological and ecological features (Henry and Wells 2010; Nygren et al. 2010; Skoracka and Dabert 2010).

Cryptic species are prevalent in taxa which are microscopic in size and have simplified bodies (Skoracka et al. 2015), and these characteristics may hinder their proper identification based on morphological techniques that are prevalent in taxonomy (de Lillo et al. 2010; Zhao et al. 2013; Stork 2018). Eriophyoid mites are characterized by their minute size and simplified body structure, and their accurate identification by morphological traits is demanding (Lindquist 1996). However, nowadays taxonomist are integrating the traditional morphology approach with molecular tools to discriminate between species and to describe new species (e.g., Chetverikov et al. 2013; de Lillo et al. 2018). This is especially important in basic and applied studies of economically important cryptic species, which may differ, for example, in capacity for pathogen transmission or pesticide resistance (Pan et al. 2011; Miller et al. 2012). Eriophyoid mites are obligatory phytophagous arthropods and are highly specialized to their hosts. They are known as significant plant pests worldwide, that cause either direct damage to their host plant or transmit plant viruses. Recent research suggests that cryptic diversity may be very common in eriophyoid mites. It was demonstrated that the grass-associated wheat curl mite (WCM), *Aceria tosichella* Keifer, Brassicaceae-associated *Aceria* mites, and coniferous-associated *Trisetacus* mites are complexes that consist of divergent lineages which probably are cryptic species (Skoracka et al. 2012; Lewandowski et al. 2014; Živković et al. 2017).

An intensively studied example of cryptic diversity in eriophyoid mites is the cereal rust mite, *Abacarus hystrix* Nalepa, that long has been regarded as a single species adapted to numerous Poaceae species, with a wide host range including both cultivated and wild grasses—e.g., *Triticum aestivum* L. (wheat), *Secale cereale* L. (rye), *Oryza sativa* L. (rice), *Cynodon dactylon* L. (Bermuda grass), *Lolium perenne* L. (perennial ryegrass), *Bromus inermis* Leyss. (smooth brome), *Bromus hordeaceus* L. (soft brome), *Bromus erectus* Huds. (meadow brome), *Dactylis glomerata* L. (cocksfoot grass) (Frost and Ridland 1996). *Abacarus hystrix* has been recognized as a common pest of cereal fields and grasslands in Eurasia, North America, temperate regions of Africa, Australia and New Zealand. Moreover, *A. hystrix* is known for its ability to transmit *Ryegrass mosaic virus* (RgMV) and *Agropyron mosaic virus* (AgMV) (Oldfield and Proeseler 1996, de Lillo and Skoracka 2010). Additionally, the mite has a capacity for rapid population growth and can build up to very high densities (Skoracka and Kuczyński 2004), what may boost mites' dispersal and infestation of new grass hosts (Sabelis and Bruin 1996). Morphological examination of the cereal rust mite populations originating from three grass species (quackgrass, ryegrass and smooth brome) demonstrated that there is an apparent and discontinuous variation of body shape, length of setae and overall body size among host-associated mite populations (Skoracka et al. 2002), which could be explained by a phenotypic adaptation to the local host environment (Pegler et al. 2005), or might indicate partial or total genetic separation (host races or species) (Drès and Mallet 2002). Further studies revealed that quackgrass- and ryegrass-associated populations differ significantly in performance and acceptance of each other's hosts. Specifically, reciprocal transfer of mite specimens between host species resulted in unsuccessful settlement on the novel host plant (Skoracka and Kuczyński 2006). Moreover, mites from different host populations quickly distinguished between their familiar host and unfamiliar hosts used by conspecific populations (Skoracka et al. 2007). All these findings suggested the narrow host specialization of studied host-populations of this putative generalist. Subsequently, pre- and post-zygotic barriers between these two populations were found, indicating the existence of reproductive isolation between them (Skoracka 2008). This pattern of sterility between ryegrass- and quackgrass-associated mites was confirmed by the differences in the mitochondrial DNA sequence of cytochrome oxidase subunit I (COI) gene and the nuclear sequence of D2 region of 28S rDNA (Skoracka and Dabert 2010). As the result of this integrative approach, which included genetic and morphometric analyses, host performance and preference tests, and reciprocal crosses, the population associated with ryegrass has been described as a new species *Abacarus lolii* Skoracka, a cryptic species of *A. hystrix* sensu lato (Skoracka 2009). Apart from the quackgrass and ryegrass populations of the cereal rust mite complex, morphometric and genetic studies included also the third population, which inhabited smooth brome (Skoracka et al. 2002; Skoracka and Dabert 2010). The analyses revealed significant discontinuous morphological variation between these three host-associated populations (Skoracka et al. 2002), which corresponded to an interspecific level of genetic variation in mtDNA COI (> 22%) (Skoracka and Dabert 2010). Moreover, the divergence in nuclear D2 region of 28S rDNA between smooth brome associated population and two other populations was > 2.5%, whereas the D2 distance of 0.2% had been experimentally designated as interspecific for *A. hystrix* sensu lato and *A. lolii* (Skoracka 2008, 2009; Skoracka and Dabert 2010). Therefore, the population from smooth brome was recognized as a new species awaiting a formal description (Skoracka and Dabert 2010).

In addition, Skoracka and Dabert (2010) observed further delineation within quackgrass- and brome-associated populations in both mitochondrial and nuclear gene fragments, wherein a well-supported split on COI gene within the brome population was

supported by differences in their amino acid sequences. Moreover, some sequences of brome-associated mites clustered on the D2 28S rDNA tree together with *Abacarus acutatus* Sukhareva, another grass-inhabiting eriophyoid species. This unresolved relationship between *A. hystrix* and *A. acutatus* led to the conclusion that the whole grass-associated *Abacarus* genus complex needs revision (Skoracka and Dabert 2010). Overall, these analyses showed that *A. hystrix* does not exist as a monophyletic taxon and the diversity of the whole grass-associated *Abacarus* genus complex may be higher than it was thought to be, whereas DNA barcoding based on mtDNA COI is a valuable tool for identification of morphologically similar eriophyoid species. Mitochondrial genes, especially COI gene, are known to be, in general, good indicators of recent diversification events, as well as a useful tool for identification at species-level resolution (Hebert et al. 2003a, b). Mitochondrial barcodes, along with variety of nuclear markers, became important supplement for animal species descriptions and taxonomic workflow (Hubert and Hanner 2015).

In this study, we continue the exploration of hidden diversity within the grass-associated *Abacarus* genus members, based on additional genetic information. Specifically, we ask what the extent of putative species boundaries within grass-associated *Abacarus* complex is and whether there is a host-associated genetic differentiation within the complex. We reveal high cryptic diversity within *Abacarus* spp. and discuss their host associations. We also proceed the story by describing the new species associated with smooth brome, on the basis of standard morphological characters and DNA barcode data (mtDNA COI and 28S rDNA D2) obtained from the type material of the new species.

Materials and methods

Sampling

Plant samples were collected mostly in Poland, but also in Finland, Lithuania and Turkey, from 18 grass species (Table 1). Plants were transported to the laboratory, where they were inspected for the presence of mites directly with a stereo-microscope. Mite specimens from the inspected leaves were transferred using an eyelash or thin insect pin glued to a dissecting needle to (1) Eppendorf tubes with 180 µL of ATL buffer (extraction buffer) (Qiagen, Hilden, Germany) for molecular analysis, and (2) microscope slides, mounted in modified Berlese medium (Monfreda et al. 2010) in dorsoventral orientation. After DNA extractions, the mite exoskeletons were also mounted on slides for additional identification.

DNA analyses

DNA was extracted according to method described by Dabert et al. (2008). The cytochrome oxidase subunit I (COI) gene fragment was amplified by PCR with the degenerate primers: bcdF01 (5'-CATTTTCHACTAAYCATAARGATATTGG-3') and bcdR04 (5'-TAT AAACYTCDGGATGNCCAAAAA-3'). PCRs were carried out in 10 µL reaction volumes containing 5 µL of Type-it Multiplex PCR Master Mix (2×MM) (Qiagen), 0.5 µM of each primer and 4 µL of DNA template using a thermocycling profile of one cycle of 5 min at 95 °C followed by 35 steps of 30 s at 95 °C, 90 s at 50 °C, 60 s at 72 °C, with a final step of 15 min at 72 °C. After amplification, reaction product was diluted twofold and 5 µL were analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide or with addition of GelRed (Biotum, Hayward, CA, USA) to assess the product size

Table 1 Characteristics of the sequences of *Abacarus* species used in this study

Mite taxon	Host plant taxon	Collection date	Country	Geographical coordinates	GenBank accession nr.
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	18-Apr-2008	Poland	52.61N, 15.44E	MG267352
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	17-Apr-2008	Poland	52.6269N, 15.8372E	MG267353
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	17-Apr-2008	Poland	52.6278N, 15.8367E	MG267354
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	17-Apr-2008	Poland	52.6303N, 15.8358E	MG267355
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	19-Apr-2008	Poland	52.6814N, 15.1725E	MG267356
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	19-Apr-2008	Poland	52.6781N, 15.1733E	MG267357
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	19-Apr-2008	Poland	52.6786N, 15.1717E	MG267358
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	20-Apr-2008	Poland	52.655N, 14.948E	MG267359
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	20-Apr-2008	Poland	52.653N, 14.947E	MG267360
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	28-Apr-2008	Poland	52.68N, 16.71E	MG267361
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	28-Apr-2008	Poland	52.77N, 16.45E	MG267362
<i>Abacarus</i> sp. 3	<i>Anthoxanthum odoratum</i>	09-May-2008	Poland	52.93N, 15.94E	MG267322
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	10-May-2008	Poland	53.08N, 15.90E	MG267363
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	10-May-2008	Poland	53.06N, 15.85E	MG267320
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	10-May-2008	Poland	53.06N, 15.85E	MG267365
<i>Abacarus acutatus</i>	<i>Calamagrostis arundinacea</i>	02-Jun-2008	Poland	53.41N, 16.87E	MG267366
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	11-Jun-2008	Poland	52.38N, 16.93E	MG267367
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	10-Jun-2008	Poland	53.25N, 16.02E	MG267368
<i>Abacarus</i> sp. 4	<i>Festuca gigantea</i>	29-Jun-2008	Poland	52.6694N, 17.5003E	MG267316
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	29-Jun-2008	Poland	52.6694N, 17.5003E	MG267317
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	29-Jun-2008	Poland	52.6694N, 17.5003E	MG267340
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	29-Jun-2008	Poland	52.6697N, 17.5008E	MG267343
<i>Abacarus</i> sp. 6	<i>Festuca heterophylla</i>	29-Jun-2008	Poland	52.6694N, 17.5003E	MG267325
<i>Abacarus</i> sp. 6	<i>Festuca heterophylla</i>	29-Jun-2008	Poland	52.6692N, 17.5008E	MG267324
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	29-Jun-2008	Poland	52.6692N, 17.5008E	MG267344
<i>Abacarus</i> sp. 4	<i>Brachypodium silvaticum</i>	29-Jun-2008	Poland	52.6694N, 17.5003E	MG267323
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	05-Aug-2008	Poland	52.5613N, 17.1142E	MG267342

Table 1 (continued)

Mite taxon	Host plant taxon	Collection date	Country	Geographical coordinates	GenBank accession nr.
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	05-Aug-2008	Poland	52.5613N, 17.1144E	MG267346
<i>Abacarus acutatus</i>	<i>Calamagrostis arundinacea</i>	05-Aug-2008	Poland	52.56N, 17.08E	MG267369
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	05-Aug-2008	Poland	52.5696N, 17.1106E	MG267370
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	08-Aug-2008	Poland	52.47N, 16.93E	MG267371
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	25-Aug-2008	Finland	60.15N, 19.56E	MG267333
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	25-Aug-2009	Finland	60.15N, 19.56E	MG267338
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	06-Aug-2012	Poland	51.85N, 17.16E	MG267332
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	06-Aug-2012	Poland	52.46N, 16.93E	MG267341
<i>Abacarus lolii</i>	<i>Lolium perenne</i>	29-Aug-2012	Poland	52.44N, 16.75E	MG267347
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	06-Aug-2012	Poland	51.85N, 17.16E	MG267348
<i>Abacarus acutatus</i>	<i>Echinochloa crus-galli</i>	06-Aug-2012	Poland	51.85N, 16.93E	MG267373
<i>Abacarus</i> sp. 2	<i>Bromus hordeaceus</i>	28-Aug-2012	Poland	52.44N, 16.75E	MG267374
<i>Abacarus</i> sp. 5	<i>Phleum pratense</i>	13-Sep-2012	Poland	51.84N, 17.16E	MG267318
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	02-Apr-2009	Poland	52.71N, 16.93E	MG267345
<i>Abacarus acutatus</i>	<i>Calamagrostis arundinacea</i>	24-Apr-2009	Poland	53.44N, 16.51E	MG267349
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	04-Jun-2009	Poland	52.06N, 16.72E	MG267329
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	04-Jun-2009	Poland	52.043N, 16.773E	MG267328
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	04-Jun-2009	Poland	52.037N, 16.77E	MG267331
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	07-Jun-2009	Poland	51.68N, 16.09E	MG267319
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	04-Jun-2009	Poland	51.68N, 16.07E	MG267321
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	04-Jun-2009	Poland	52.22N, 17.22E	MG267326
<i>Abacarus hystrix</i>	<i>Triticosecale</i>	16-Jun-2009	Poland	52.73N, 17.76E	MG267334
<i>Abacarus hystrix</i>	<i>Triticosecale</i>	17-Jul-2009	Poland	52.03N, 16.76E	MG267335
<i>Abacarus hystrix</i>	<i>Triticosecale</i>	17-Jul-2009	Poland	52.03N, 16.78E	MG267330
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	15-Jul-2009	Poland	51.95N, 16.28E	MG267337

Table 1 (continued)

Mite taxon	Host plant taxon	Collection date	Country	Geographical coordinates	GenBank accession nr.
<i>Abacarus hystrix</i>	<i>Hordeum murinum</i>	15-Jul-2009	Poland	51.96N, 16.25E	MG267336
<i>Abacarus acutatus</i>	<i>Calamagrostis arundinacea</i>	25-Aug-2009	Lithuania	55.77N, 25.80E	MG267364
<i>Abacarus acutatus</i>	<i>Calamagrostis arundinacea</i>	25-Aug-2009	Lithuania	55.79N, 25.76E	MG267350
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	21-Aug-2009	Finland	59.92N, 20.92E	MG267351
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	21-Aug-2009	Finland	59.92N, 20.90E	MG267372
<i>Abacarus hystrix</i>	<i>Elymus hispidus</i>	30-Aug-2009	Turkey	39.70N, 44.12E	MG267327
<i>Abacarus</i> sp. 7	<i>Bromus inermis</i>	24-May-2010	Poland	52.91N, 17.45E	MG267339
<i>Abacarus</i> sp. 7	<i>Bromus inermis</i>	13-Jan-2007	Poland	52.27N, 16.56E	FJ387556
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	17-Mar-2007	Poland	52.11N, 17.55E	FJ387557
<i>Abacarus plumiger</i>	<i>Bromus inermis</i>	18-Mar-2007	Poland	52.11N, 17.57E	FJ387558
<i>Abacarus</i> sp. 7	<i>Bromus inermis</i>	10-Jun-2007	Poland	52.06N, 16.39E	FJ387559
<i>Abacarus longilobus</i>	<i>Bromus erectus</i>	03-Jul-2009	Turkey	37.32N, 43.45E	KC412846
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	14-Jun-2007	Poland	52.14N, 16.43E	FJ387542
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	13-Jan-2007	Poland	52.45N, 16.90E	FJ387543
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	08-Jun-2007	Poland	52.22N, 16.56E	FJ387544
<i>Abacarus acutatus</i>	<i>Calamagrostis epigejos</i>	10-Jun-2007	Poland	51.65N, 16.10E	FJ387545
<i>Abacarus longilobus</i>	<i>Dactylis glomerata</i>	24-Jul-2009	Turkey	39.19N, 43.88E	KC412845
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	02-Feb-2007	Poland	52.27N, 16.54E	FJ387546
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	17-Feb-2007	Poland	52.22N, 16.56E	FJ387547
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	10-Mar-2007	Poland	52.20N, 16.52E	FJ387548
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	10-Jun-2007	Poland	51.49N, 16.20E	FJ387549
<i>Abacarus hystrix</i>	<i>Elymus repens</i>	27-Oct-2006	Poland	52.28N, 16.55E	FJ387550
<i>Abacarus hystrix</i>	<i>Elymus hispidus</i>	30-Aug-2009	Turkey	38.45N, 43.40E	KC412843
<i>Abacarus lolii</i>	<i>Lolium perenne</i>	10-Jan-2007	Poland	52.22N, 16.55E	FJ387551
<i>Abacarus lolii</i>	<i>Lolium perenne</i>	10-Apr-2007	Poland	52.22N, 16.54E	FJ387552

Table 1 (continued)

Mite taxon	Host plant taxon	Collection date	Country	Geographical coordinates	GenBank accession nr.
<i>Abacarus loli</i>	<i>Lolium perenne</i>	09-Jun-2007	Poland	51.65N, 16.10E	FJ387553
<i>Abacarus loli</i>	<i>Lolium perenne</i>	10-Jun-2007	Poland	51.39N, 15.53E	FJ387554
<i>Abacarus loli</i>	<i>Lolium perenne</i>	28-Oct-2006	Poland	52.28N, 16.56E	FJ387555
<i>Abacarus hystrix</i>	not available	Not available	China	not available	KM111087
<i>Abacarus hystrix</i>	<i>Triticum aestivum</i>	03-Jul-2010	Turkey	39.56N, 44.01E	KC412844

and concentration. Then, the sample was either sequenced directly in both directions using 1–5 µL of the PCR product and 50 pmol of PCR primer, or after an enzymatic clean-up, using 15 pmol of primer and approximately 30 ng of PCR product. Amplification of the ca. 600 bp D2 region in 28S rDNA was performed using the primers D1D2fw2 (Sonnenberg et al. 2007) and 28SR0990 (Mironov et al. 2012) and followed the same protocol as COI amplification reaction, except for primers amount, which was 0.25 µM each. Products were sequenced in both directions with PCR primers and with specific sequencing primers Er28SF05 and Er28SR05 (Szydio et al. 2015). Sequencing was performed using BigDye Terminator v.3.1 chemistry (Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). Trace files were checked and edited using MEGA6 (Tamura et al. 2013).

Newly generated 59 mitochondrial *Abacarus* COI sequences were combined with 23 previously published in NCBI GenBank database (till August 2017) COI sequences of grass-associated *Abacarus* (Skoracka and Dabert 2010; Szydio et al. 2015; Xue et al. 2017) and outgroup *Trisetacus silvestris* Castagnoli data (GenBank Acc. No. KC776553.1; Lewandowski et al. 2014) for analysis (Table 1). Sequences were aligned using the ClustalW algorithm (Higgins et al. 1996) implemented in MEGA6, and the final datasets contained 82 *Abacarus* COI sequences (603 bp) of mites from 18 identified grass species, one unknown grass species and five countries (Table 1).

Alignment reliability and product authenticity of COI sequences was validated by translating aligned DNA sequences into amino acids and assessing the alignments for premature stop codons. For COI dataset, neighbor-joining (NJ) tree with 1000 bootstrap replicates was constructed using the Kimura 2-parameter model (Kimura 1980). Additionally, maximum likelihood (ML) analysis was done using GTR+G+I model as selected by Modeltest v.2.1.3 (Guindon and Gascuel 2003; Darriba et al. 2012) according to the Akaike and Bayesian information criteria (AIC, BIC).

The D2 sequence of the new species was aligned with corresponding sequences from the study by Skoracka and Dabert (2010) that fell into to the clade investigated as a putative species of *Abacarus* complex from smooth brome, and with *Abacarus longilobus* Skoracka, a sister species of the new species. These sequences were downloaded from Genbank (FJ392672, FJ392673, FJ392674, KC776553, respectively).

Pairwise mean genetic distances between clades were calculated for COI and D2 datasets with the Kimura 2-parameter model (K2P) (Kimura 1980). Mean K2P genetic distances within clades were calculated for COI dataset, and standard error estimates were obtained by a bootstrap procedure (1000 replicates). All above analyses were done in MEGA6. To confirm the presence of *Abacarus* species we applied the combination of the Poisson Tree Processes model for species delimitation (PTP) and a Bayesian implementation of PTP (bPTP). This approach allows to infer putative species boundaries on a given phylogenetic input tree (Zhang et al. 2013). The analysis was done with bPTP server (bPTP server: a Bayesian implementation of the PTP model for species delimitation, <http://species.h-its.org/ptp/>). The input tree for bPTP model was obtained by Bayesian inference approach with codon partitioning. The Hasegawa-Kishino-Yano (Hasegawa et al. 1985) substitution model with proportion of invariable sites (I) (Shoemaker and Fitch 1989) and gamma distributed rate of variation among sites (G) (Yang 1994) (HKY+I+G) was applied to the first and second codon positions in sequences, and General Time Reversible model (Tavaré 1986) with I and G (GTR+I+G) was applied to the third position of each codon. The analysis was run with MrBayes v.3.2 software (Ronquist et al. 2012) as two parallel runs with four chains (three heated and one cold chain), with G and I estimated during the analysis for each model. The analysis was run for 560,000 generations, until the

Fig. 1 Neighbor-joining tree constructed using the Kimura 2-parameter model for mtDNA COI sequences of the *Abacarus* complex and outgroup species. Values above the branches represent bootstrap-measured support (1000 replicates) equal to or higher than 60. Maximum likelihood analysis resulted in concordant topology in defining species groups. Colors represent different genetic lineages/species: yellow—*Abacarus acutatus*; azure—*Abacarus* sp. collected in Turkey; blue—*Abacarus hystrix* complex 1 and 2; brown—*Abacarus* sp. 1; burgundy—*Abacarus* sp. 2; red—*Abacarus plumiger* n. sp.; green—*Abacarus longilobus*; pink—*Abacarus* sp. 3; lilac—*Abacarus* sp. 4; light brown—*Abacarus* sp. 5; dark yellow—*Abacarus* sp. 6; navy blue—*Abacarus lolii*; purple—*Abacarus* sp. 7. Symbols on the tips of branches correspond to host plant species. (Color figure online)

average standard deviation of split frequencies value for runs was less than 0.01. When the convergence was reached, the analysis was stopped, 25% of first trees were discarded from results, and consensus tree with posterior probabilities was generated. The Monte Carlo sampler for the PTP model was applied to the tree with following parameters: 200,000 generations; thinning: 100; burnin: 0.1 and seed: 123.

Morphological analyses

Mites were studied with a phase-contrast microscope Olympus BX41. All specimens were taxonomically identified on the basis on apparent morphological characteristics as belonging to *Abacarus* genus complex (Sukhareva 1985; Nalepa 1896; Skoracka et al. 2002). Specimens of a newly described species were measured. Morphological nomenclature follows Lindquist (1996), data measurements follow Amrine and Manson (1996), and systematic classification follows Amrine et al. (2003). Measurements are given in micrometers. Measurements refer to the lengths of each structure unless otherwise stated. Positions of leg setae were measured from the proximal margin of the seta-bearing segment. Locations of ventral setae *c2*, *d*, *e*, and *f* on ventral annuli were measured from the posterior margin of coxae II. The length of prodorsal shield was measured from the anterior margin of frontal lobe to the posterior margin of shield. The distance between setae was measured as the distance between the center of tubercles. The holotype female measurement precedes the corresponding range for paratypes (given in parentheses). For adult males and nymph instar only the ranges are given. The means of the measurements are given in Table 3. Scanning electron micrographs (SEM) were taken in the Institute of Plant Protection, Poznań, Poland using Hitachi S3000N, uncoated specimen photographed at -20°C and 60 Pa.

Results

Genetic differentiation within the *Abacarus* complex

The final COI dataset consisted of 83 aligned sequences which represented 82 ingroup populations of grass-associated *Abacarus* mites (Table 1), and one outgroup. The 78 sequences of *Abacarus* formed 10 highly supported clades (bootstrap value: 99%) and four remaining sequences represented distinct genetic lineages (Fig. 1). Relationships between lineages were not fully resolved. The genetic distances between lineages ranged from 6.6 to 26.5% (Table 2). The PTP/bPTP analyses' results were consistent with NJ structuring and identified 14 lineages as putative species (Fig. 2).

The NJ analysis of partial COI gene confirmed the monophyly of four already described species within the complex: *A. lolii*, *A. longilobus*, *A. hystrix* and *A. acutatus*. Additionally,

Host legend

- ▲ - *Calamagrostis epigejos*
- ▼ - *Echinochloa crus-galli*
- △ - *Calamagrostis arundinacea*
- ▽ - *Elymus hispidus*
- ▶ - *Elymus repens*
- - *Triticosecale*
- - *Triticum aestivum*
- ◀ - *Hordeum murinum*
- ◄ - *Bromus mollis*
- ◁ - *Bromus inermis*
- ◀ - *Dactylis glomerata*
- ◁ - *Bromus erectus*
- ◀ - *Anthoxanthum odoratum*
- ◁ - *Festuca gigantea*
- ◀ - *Brachypodium silvaticum*
- ◁ - *Phleum pratense*
- ◀ - *Festuca heterophylla*
- ◁ - *Lolium perenne*
- * - samples collected outside Poland

Table 2 Within-group Kimura 2-parameter (K2P) distance (%) with standard errors in parentheses (diagonal), and between-group K2P distance (%) below the diagonal, with standard errors above the diagonal

Group	<i>Abac- arius acutatus</i>	<i>Abac- arius hystrix</i> complex	<i>Abac- arius hystrix</i> complex	<i>Abac- arius loli</i>	<i>Abac- arius longilo- bus</i>	<i>Abac- arius plumiger</i> n. sp.	<i>Abac- arius</i> coll. in Turkey	<i>Abac- arius</i> sp. 1	<i>Abac- arius</i> sp. 2	<i>Abac- arius</i> sp. 3	<i>Abac- arius</i> sp. 4	<i>Abac- arius</i> sp. 5	<i>Abac- arius</i> sp. 6	<i>Abac- arius</i> sp. 7	Outgroup
<i>Abacarus acutatus</i>	6.7 (0.6)	2.1	2.2	2.2	2.2	2.2	2.1	2.1	2.0	2.3	2.1	2.1	2.3	2.2	2.9
<i>Abacarus hystrix</i> complex 1	22.9	0.2 (0.2)	1.9	2.0	2.3	2.4	2.1	2.2	2.2	2.2	2.1	2.1	2.1	2.2	2.8
<i>Abacarus hystrix</i> complex 2	24.6	18.1	1.1 (0.2)	2.4	2.3	2.2	2.1	2.3	2.3	2.1	2.2	2.2	2.1	2.5	2.8
<i>Abacarus loli</i>	24.5	20.4	23.5	0.4 (0.2)	2.3	2.3	2.0	2.2	2.1	1.4	1.2	1.3	1.1	2.3	3.1
<i>Abacarus longilo- bus</i>	25.9	26.3	23.9	22.9	0.2 (0.2)	1.1	2.6	2.4	2.3	2.1	2.1	2.2	2.2	2.3	3.2
<i>Abacarus plu- miger</i> n. sp.	25.3	25.9	23.7	22.4	6.9	0.8 (0.2)	2.4	2.5	2.2	2.1	2.2	2.2	2.2	2.3	2.9
<i>Abacarus</i> sp. coll. in Turkey	21.9	19.2	21.0	18.6	26.1	24.5	n/a	2.5	2.2	2.1	1.8	1.8	1.9	2.1	3.1
<i>Abacarus</i> sp. 1	23.3	23.8	23.8	23.9	25.8	25.6	26.6	n/a	2.2	2.1	2.1	2.0	2.2	2.1	3.0
<i>Abacarus</i> sp. 2	23.1	22.6	23.2	20.8	23.1	22.2	22.1	22.8	n/a	2.2	2.2	2.3	2.3	2.2	2.9

Table 2 (continued)

Group	<i>Abac- arius acutatus</i>	<i>Abac- arius hystrix</i> complex 1	<i>Abac- arius hystrix</i> complex 2	<i>Abac- arius lolii</i>	<i>Abac- arius longilo- bus</i>	<i>Abac- arius plumiger</i> n. sp.	<i>Abac- arius</i> sp. coll. in Turkey	<i>Abac- arius</i> sp. 1	<i>Abac- arius</i> sp. 2	<i>Abac- arius</i> sp. 3	<i>Abac- arius</i> sp. 4	<i>Abac- arius</i> sp. 5	<i>Abac- arius</i> sp. 6	<i>Abac- arius</i> sp. 7	Outgroup
<i>Abacarus</i> sp. 3	24.6	21.9	20.1	10.3	21.9	20.9	19.7	22.5	21.3	n/a	1.5	1.5	1.3	2.3	2.8
<i>Abacarus</i> sp. 4	22.6	20.3	21.6	8.0	20.5	22.0	16.2	22.1	21.5	10.8	0.3 (0.2)	1.2	1.2	2.3	3.0
<i>Abacarus</i> sp. 5	24.4	19.4	21.1	8.2	21.8	21.9	16.4	21.4	22.2	11.7	7.8	n/a	1.2	2.2	3.1
<i>Abacarus</i> sp. 6	25.1	20.4	21.6	6.6	21.7	22.5	17.2	22.9	22.2	10.0	7.4	8.4	0.2 (0.2)	2.2	2.9
<i>Abacarus</i> sp. 7	24.5	21.1	25.4	22.3	23.1	23.9	21.7	22.7	22.8	23.2	23.4	22.2	22.4	0.7 (0.3)	2.8
outgroup	37.7	37.0	34.3	37.4	39.2	36.6	36.6	35.6	34.5	33.8	35.5	38.1	35.0	35.4	n/a

Fig. 2 Combined Poisson Tree Processes model for species delimitation (PTP) and a Bayesian implementation of PTP (bPTP) confirming putative species groups within the *Abacarus* complex and identifying additional putative species groups within *A. hystrix* complex 2 and *A. acutatus*. Blue lines indicate the unique species groups and red clades indicate species groups containing more than one haplotypes. Numbers above the branches are Bayesian posterior probabilities; only values >0.8 are shown. Vertical lines are marked to species: yellow—*Abacarus acutatus*; azure—*Abacarus* sp. collected in Turkey; blue—*Abacarus hystrix* complex 1 and 2; brown—*Abacarus* sp. 1; burgundy—*Abacarus* sp. 2; red—*Abacarus plumiger* n. sp.; green—*Abacarus longilobus*; pink—*Abacarus* sp. 3; lilac—*Abacarus* sp. 4; light brown—*Abacarus* sp. 5; dark yellow—*Abacarus* sp. 6; navy blue—*Abacarus lollii*; purple—*Abacarus* sp. 7. Genbank accession numbers are shown on the branches. (Color figure online)

high genetic differentiation was detected within *A. hystrix* and *A. acutatus*, with the mean ($\pm$ SE) level of intra-clade genetic variation at $6.1 \pm 0.7\%$ and $6.7 \pm 0.6\%$, and additional delineation detected within these taxa by PTP/bPTP analysis (Fig. 2). Within *A. hystrix*, two clades are apparent (*Abacarus hystrix* 1 and *Abacarus hystrix* 2, each with the support of 99%) with the COI distance between them of $18.1 \pm 1.9\%$. Maximum likelihood analysis resulted in concordant topology in defining species groups. The PTP/bPTP analysis revealed one additional clade (putative species) within *Abacarus hystrix* 2 and seven clades within *A. acutatus* (Fig. 2).

All analyses (genetic trees and distances) confirmed the divergence of five other clades (*Abacarus* sp. collected in Turkey, *Abacarus* sp. 4, 6, and 7, and *A. plumiger*, described during this study) and four lineages represented by single sequences (*Abacarus* sp. 1, 2, 3, and 5).

The pattern of host-associated differentiation was observed within the complex (Fig. 1). *Abacarus acutatus* was associated predominantly with plants from *Calamagrostis* genus, and one population was found on *Echinochloa crus-galli* (L.) P.Beauv. *Abacarus hystrix* group (both *Abacarus hystrix* 1 and *Abacarus hystrix* 2) was the most polyphagous taxon, found on quackgrass, wall barley, wheat and triticale. It was the only lineage associated with cereals. *Abacarus* sp. 1 was represented by the sequence from a mite population collected from an unknown host plant in China, which had been identified by the authors as *A. hystrix* (GenBank Acc. No. KM111087). However, it is highly divergent from other *Abacarus* sequences, including *A. hystrix* sensu lato ($> 21\%$). *Abacarus* sp. 2, found on *Bromus hordeaceus* (soft brome), also represented a divergent lineage ($> 20\%$). The new *Abacarus* species (*plumiger*) collected from *Bromus inermis* (smooth brome) was a sister clade (99% bootstrap support) to *A. longilobus* clade, a species associated with *Dactylis glomerata* (cocksfoot grass) and *Bromus erectus* (meadow brome). Interestingly, smooth brome was also host to another species, highly diverged from *A. plumiger* (mean $\pm$ SE = $23.9 \pm 2.3\%$). *Abacarus lolii* was associated exclusively with *Lolium perenne* (perennial ryegrass), and its sequences formed a highly supported clade. The analyses pointed out four more distinct *Abacarus* lineages that were associated with plants of the genera *Festuca*, *Brachypodium*, *Phleum*, and *Anthoxanthum* (Fig. 1).

New species within the *Abacarus* complex

Superfamily ERIOPHYOIDEA Nalepa, 1898.

Family ERIOPHYIDAE Nalepa, 1898.

Subfamily PHYLLOCOPTINAE Nalepa, 1892.

Tribe ANTHOCOPTINI Amrine and Stasny, 1994.

Genus *Abacarus* Keifer, 1944.

Abacarus plumiger n. sp. Laska, Majer, Szydło & Skoracka (Figs. 3, 4; Table 3).

Female (holotype and 29 paratypes)—Body spindleform with narrow middle dorsal ridge in opisthosoma, reaching $\frac{3}{4}$ part of the body. Body 257 (209–329); width 56 (52–59). Gnathosoma curved downward, 27 (26–29), dorsal pedipalpal genual setae *d* 12 (11–13), setae *ep* 3 (3), cheliceral stylets 21 (17–22). Prodorsal shield subtriangular, 56 (51–60), 48 (43–53) wide, with frontal lobe over base of chelicerae. Shield pattern: median line absent; admedian lines entire, reach submedian lines anteriorly; submedian subparallel to lateral margin of shield. Tubercles of setae *sc* at rear shield margin, basal axes transverse, setae *sc* 27 (21–36), 31 (26–35) apart. Legs with all usual segments

Fig. 3 *Abacarus plumiger* n. sp. female: AL—antero-lateral view; PL—postero-lateral view; em—empodium (enlarged); IG—internal genitalia (enlarged); AD—antero-dorsal view; CG—coxisternal and genital region; male: GM—genital region. Scale bars are 10 μ m

and setae present. Leg *I* 39 (37–40); femur 12 (10–12), setae *bv* 18 (16–19); genu 7 (6–7), setae *l''* 35 (35–39); tibia 10 (10), setae *l'* 12 (10–12); tarsus 8 (7–10), setae: *ft''* 23 (2–27), *ft'* 21 (19–22), *u'* 7 (6–8); solenidion *w* 11 (10–12); empodium 12 (10–12), simple, bilaterally symmetrical, with 8 (8–9) paired rays. Leg *II* 33 (33–36); femur 10 (10–11), setae *bv* 19 (14–24); genu 6 (5–7), setae *l''* 16 (13–19); tibia 8 (7–9); tarsus 9 (8–10), setae *ft''* 33 (26–32), *ft'* 12 (10–13), *u'* 7 (6–8); solenidion *w* 12 (10–12); empodium 11 (10–12), simple, bilaterally symmetrical, with 8 (8–9) paired rays. Coxal plates with irregular dashes. Setae *Ib* 9 (8–10), 12 (10–14) apart; setae *Ia* 41 (38–42), 10 (8–10) apart; setae *2a* 57 (46–65), 29 (26–29) apart; distance between setae *Ib* and *Ia* 7 (7–8), distance between setae *Ia* and *2a* 9 (8–10). Genital opening 17 (14–18), 25

Fig. 4 *Abacarus plumiger* n. sp. scanning electron micrographs. Scale bars are 10 µm. **a** Female, dorsal view; **b** male, latero-dorsal view

(22–28) wide, genital coverflap semicircular, 17 (14–18) long, 25 (22–28) wide, with 13 (13–14) longitudinal ridges. Internal genitalia: oval spermathecae, filamentous spermathecal tubes, and trapezoidal anterior genital apodeme with 13 (13–14) longitudinal ridges; setae *3a* 48 (46–64), 19 (16–22) apart. Opisthosoma with 60th (60th–74th) dorsal annuli with minute conical microtubercles, pointed on the posterior annuli; 76 (70–90) ventral annuli with minute microtubercles, 7 (6–7) semiannuli between coxae and genital coverflap. Setae *c2* 48 (43–59), 55 (50–57) apart, on 8th (7th–9th) annulus; setae *d* 50 (40–72), 31 (32–36) apart, on 20th (19th–21st) annulus; setae *e* 48 (36–61), 13 (13–15) apart, on 40th (40th–44th) annulus; setae *f* 35 (27–45), 22 (20–23) apart, on 71st (65th–84th) annulus, 5th (5th–6th) annulus from rear. Setae *h1* 8 (6–9), 10 (6–10) apart; setae *h2* 102 (78–110), 12 (10–12) apart; distance between *h1* and *h2* 2 (2).

Male ($n=11$)—Body (180–228) spindleform with narrow middle dorsal ridge in opisthosoma, reaching $\frac{3}{4}$ part of the body; width (53–59). Gnathosoma curved downward, (23–27), dorsal pedipalpal genual setae *d* (10–12), setae *ep* 3 (2–3), cheliceral stylets (11–17). Prodorsal shield subtriangular, (44–50), (40–46) wide, with frontal lobe over base of chelicerae. Shield pattern: median line absent; admedian lines entire, reach submedian lines anteriorly; submedian subparallel to lateral margin of shield. Tubercles of setae *sc* at rear shield margin, basal axes transverse, (24–29) apart, setae *sc* (15–25). Legs with all usual segments and setae present. Leg *I* (31–36); femur (9–11), setae *bv* (11–15); genu (5–7), setae *l''* (26–32); tibia (6–8), setae *l'* (9–13); tarsus (7–8), setae *ft''* (25–30), *ft'* (20–28), *u'* (6–8); solenidion *w* (8–11); empodium (7–10), simple, bilaterally symmetrical, with 8 (8–9) paired rays. Leg *II* (28–33); femur (9–11), setae

Table 3 Morphological measurements (μm) of females, males, and nymphs of *Abacarus pulmiger* n. sp

Trait	Female					Male					Nymph				
	n	mean	SD	min	max	n	mean	SD	min	max	n	mean	SD	min	max
Length of body	30	278.1	27.1	209	329	11	202.7	13.6	180	228	5	184.6	16.8	167	204
Width of body	30	54.9	1.8	52	59	11	57.5	2.0	53	59	5	50.0	2.2	47	53
Length of gnathosomal setae <i>d</i>	30	12.2	0.7	11	13	11	11.2	0.9	10	12	5	8.8	0.4	8	9
Length of gnathosomal setae <i>cp</i>	16	3.0	0.0	3	3	6	2.4	0.5	2	3	2	2.0	0.0	2	2
Length of chelicerae	30	20.2	1.3	17	22	11	13.8	1.7	11	17	5	15.2	2.9	11	19
Length of gnatosoma	30	27.9	0.9	26	29	10	25.6	1.6	23	27	4	19.8	2.8	17	23
Length of prodorsal shield	30	55.6	2.2	51	60	11	47.4	2.0	44	50	5	41.4	2.4	38	44
Width of prodorsal shield	30	49.9	1.6	48	53	11	43.4	1.7	40	46	5	37.6	1.5	36	40
Length of setae <i>sc</i>	30	27.3	4.0	21	36	11	20.4	3.7	15	25	5	10.0	0.7	9	11
Setae <i>sc</i> apart	30	31.7	2.2	26	35	11	27.5	1.9	24	29	5	20.8	1.3	19	22
No. of dorsal annuli	30	65.7	3.2	60	74	11	61.0	8.6	52	73	4	70.0	2.2	68	73
No. of ventral annuli	30	77.9	3.9	70	90	11	65.6	6.0	57	74	2	65.0	0.0	65	65
No. of semiannuli between coxae and genital coverflap	20	6.5	0.5	6	7	–	–	–	–	–	–	–	–	–	–
Length of setae <i>c2</i>	30	51.3	3.7	43	59	11	46.4	3.4	40	51	5	30.8	4.1	25	35
Setae <i>c2</i> apart	30	53.1	1.7	50	57	10	54.3	2.3	50	56	5	48.2	3.1	45	52
No. of annuli of setae <i>c2</i>	30	8.5	0.5	8	9	11	8.7	0.9	8	10	2	9.5	0.7	9	10
Length of setae <i>d</i>	30	55.4	7.3	40	72	11	47.0	4.0	41	52	5	26.6	2.7	23	30
Setae <i>d</i> apart	30	33.6	1.4	31	36	10	34.4	1.8	32	37	5	27.8	3.0	24	30
No. of annuli of setae <i>d</i>	30	20.0	0.7	19	21	11	20.4	1.4	18	23	2	23.5	0.7	23	24
Length of setae <i>e</i>	30	46.8	7.1	36	61	10	29.7	2.1	25	33	5	12.6	0.9	11	13
Setae <i>e</i> apart	30	14.2	0.7	13	15	11	14.4	1.4	12	16	5	12.8	0.8	12	14
No. of annuli of setae <i>e</i>	30	41.7	1.2	40	44	11	36.1	3.2	31	40	2	37.5	0.7	37	38
Length of setae <i>f</i>	30	34.9	3.8	27	45	10	35.6	3.1	32	41	5	23.6	1.3	22	25
Setae <i>f</i> apart	30	21.9	1.0	20	23	10	24.1	2.0	21	26	5	19.6	2.1	17	22
No. of annuli of setae <i>f</i>	30	72.7	3.9	65	84	11	61.0	5.6	52	68	2	60.5	0.7	60	61

Table 3 (continued)

Trait	Female					Male					Nymph				
	n	mean	SD	min	max	n	mean	SD	min	max	n	mean	SD	min	max
No. of annuli of setae <i>f</i> from rear	30	5.2	0.4	5	6	11	5.2	0.4	5	6	4	5.3	0.5	5	6
Length of setae <i>h1</i>	30	8.0	1.0	6	9	11	6.0	0.4	5	7	5	3.6	0.5	3	4
Length of setae <i>h2</i>	20	94.3	6.9	78	110	7	82.4	13.9	62	102	4	63.3	6.2	55	70
Setae <i>h1</i> apart	23	7.9	1.1	6	10	10	6.7	0.8	5	8	5	6.0	0.0	6	6
Setae <i>h2</i> apart	19	10.9	0.9	10	12	11	11.1	1.5	7	12	5	9.6	0.5	9	10
Setae <i>h1</i> and <i>h2</i> apart	29	2.0	0.0	2	2	11	2.8	0.6	2	4	5	2.0	0.0	2	2
Length of external genitalia	30	17.0	0.9	14	18	11	12.6	1.0	11	14	–	–	–	–	–
Width of external genitalia	30	25.0	1.2	22	28	11	21.0	0.6	20	22	–	–	–	–	–
Length of setae <i>3a</i>	30	47.8	6.2	36	64	11	28.3	2.1	25	32	5	12.6	2.6	10	17
Setae <i>3a</i> apart	30	18.1	1.1	16	22	11	17.7	0.6	17	19	5	9.6	0.5	9	10
No. of ridges on genital coverflap	30	12.3	1.9	3	14	–	–	–	–	–	–	–	–	–	–
Setae <i>1b</i> apart	30	11.7	0.8	10	14	10	10.4	1.0	10	13	4	10.0	0.0	10	10
Length of setae <i>1b</i>	30	9.1	0.7	8	10	10	8.9	1.0	8	10	4	6.8	1.5	5	8
Setae <i>1a</i> apart	30	9.5	0.6	8	10	11	8.4	1.8	7	13	5	8.0	0.7	7	9
Length of setae <i>1a</i>	28	40.4	1.3	38	42	11	22.5	3.1	18	27	5	13.4	0.9	12	14
Setae <i>2a</i> apart	30	27.7	1.0	26	29	11	22.7	1.4	21	26	5	23.6	1.7	22	26
Length of setae <i>2a</i>	30	55.8	5.2	46	65	8	40.8	5.2	33	47	5	33.6	5.0	30	40
Setae <i>1b</i> and <i>1a</i> apart	29	7.4	0.5	7	8	10	7.4	0.8	6	9	5	7.8	0.4	7	8
Setae <i>1a</i> and <i>2a</i> apart	28	8.9	0.4	8	10	11	8.5	0.5	8	9	5	9.0	0.7	8	10
Length of leg 1	27	38.1	0.9	37	40	11	32.6	1.8	31	36	5	25.0	0.7	24	26
Length of femur	30	11.4	0.7	10	12	11	10.3	0.6	9	11	5	8.0	1.2	7	10
Length of setae <i>bv</i>	29	17.3	0.9	16	19	11	12.5	1.6	11	15	5	8.6	1.1	7	10
Length of genu	30	6.5	0.5	6	7	11	6.4	0.7	5	7	5	4.4	0.5	4	5
Length of setae <i>1''</i>	30	37.0	1.3	35	39	11	28.5	2.0	26	32	5	24.4	1.7	23	27

Table 3 (continued)

Trait	Female				Male				Nymph						
	n	mean	SD	min	max	n	mean	SD	min	max	n	mean	SD	min	max
Length of tibia	30	10.0	0.5	10	10	11	6.6	0.7	6	8	5	4.2	0.4	4	5
Length of setae <i>l'</i>	27	11.3	0.7	10	12	11	10.7	1.3	9	13	5	7.2	0.4	7	8
Length of tarsus	30	8.4	0.5	7	10	11	7.7	0.5	7	8	5	6.4	0.5	6	7
Length of setae <i>fl''</i>	29	24.4	4.5	2	27	10	28.2	1.9	25	30	5	19.6	1.5	17	21
Length of setae <i>fl'</i>	29	20.3	0.8	19	22	9	25.0	2.8	20	28	5	18.0	2.3	15	20
Length of setae <i>u'</i>	29	7.2	0.6	6	8	11	7.2	0.6	6	8	5	5.4	0.9	5	7
Length of solenidion	30	10.8	0.7	10	12	11	9.8	1.0	8	11	5	9.0	0.0	9	9
Length of empodium	30	11.0	0.5	10	12	9	8.3	1.0	7	10	5	6.4	0.5	6	7
Length of leg 2	30	34.0	0.9	33	36	11	30.5	1.2	28	33	5	24.4	1.1	23	26
Length of femur	29	10.6	0.5	10	11	11	9.9	0.5	9	11	5	7.4	0.5	7	8
Length of setae <i>b_v</i>	27	19.2	3.0	14	24	11	22.1	1.6	19	25	5	13.2	1.1	12	15
Length of genu	30	6.0	0.7	5	7	11	5.7	0.6	5	7	5	4.4	0.5	4	5
Length of setae <i>l''</i>	25	16.0	1.4	13	19	11	14.3	1.5	12	16	5	12.0	1.7	11	15
Length of tibia	30	7.7	0.4	7	9	11	5.8	0.6	5	7	5	4.2	0.4	4	5
Length of tarsus	30	8.5	0.4	8	10	11	7.2	0.9	6	8	5	5.6	0.5	5	6
Length of setae <i>fl''</i>	28	28.7	1.7	26	33	11	26.6	1.7	24	30	5	19.8	1.6	18	22
Length of setae <i>fl'</i>	28	11.4	1.0	10	13	10	10.7	1.2	9	12	4	7.0	0.8	6	8
Length of setae <i>u'</i>	29	6.9	0.7	6	8	10	6.8	0.8	6	8	5	4.2	0.4	4	5
Length of solenidion	30	10.8	0.7	10	12	11	10.4	0.8	9	11	5	8.6	0.5	8	9
Length of empodium	30	11.0	0.6	10	12	11	8.4	1.1	7	11	5	6.0	0.0	6	6
No. of rays—empodium 1	30	8.3	0.5	8	9	9	8.0	0.0	8	8	5	7.2	0.4	7	8
No. of rays—empodium 2	29	8.2	0.4	8	9	11	8.0	0.0	8	8	5	7.2	0.4	7	8

bv (19–25); genu (5–7), setae *l''* (12–16); tibia (5–7); tarsus (6–8), setae *ft''* (24–30), *ft'* (9–12), *u'* (6–8); solenidion *w* (9–11); empodium (7–11), simple, bilaterally symmetrical, with 8 (8–9) paired rays. Coxal plates with irregular dashes. Setae *lb* (8–10), (10–13) apart; setae *la* (18–27), (7–13) apart; setae *2a* (33–47), (21–26) apart; distance between setae *lb* and *la* (6–9), distance between setae *la* and *2a* (8–9). Genital opening (11–14), (20–22) wide; setae *3a* (25–32), (17–19) apart. Opisthosoma with (52nd–73rd) dorsal annuli with minute conical microtubercles, pointed on the posterior annuli; (57–74) ventral annuli with minute microtubercles. Setae *c2* (40–51), (50–56) apart, on (8th–10th) annulus; setae *d* (41–52), (32–37) apart, on (18th–23rd) annulus; setae *e* (25–33), (12–16) apart, on (31st–40th) annulus; setae *f* (32–41), (21–26) apart, on (52nd–68th) annulus, (5th–6th) annulus from rear. Setae *h1* (5–7), (5–8) apart; setae *h2* (62–102), (7–12) apart; distance between *h1* and *h2* (2–4).

Nymph (n=5)—Body (167–204); width (47–53). Gnathosoma curved downward, (17–23), dorsal pedipalpal genual setae *d* (8–9), setae *ep 2* (2), cheliceral stylets (11–19). Prodorsal shield subtriangular, (38–44), (36–40) wide, with frontal lobe over base of chelicerae. Shield pattern: median line absent; admedian lines entire, reach submedian lines anteriorly; submedian subparallel to lateral margin of shield. Tubercles of setae *sc* at rear shield margin, basal axes transverse, (19–22) apart, setae *sc* (9–11). Legs with all usual segments and setae present. Leg *I* (24–26); femur (7–10), setae *bv* (7–10); genu (4–5), setae *l''* (23–27); tibia (4–5), setae *l'* (7–8); tarsus (6–7), setae *ft''* (17–21), *ft'* (15–20), *u'* (5–7); solenidion *w* (9); empodium (6–7), simple, bilaterally symmetrical, with 8 (8–9) paired rays. Leg *II* (23–26); femur (7–8), setae *bv* (12–15); genu (4–5), setae *l''* (11–15); tibia (4–5); tarsus (5–6), setae *ft''* (18–22), *ft'* (6–8), *u'* (4–5); solenidion *w* (8–9); empodium (6), simple, bilaterally symmetrical, with 8 (8–9) paired rays. Coxal plates with irregular dashes. Setae *lb* (5–8), (10) apart; setae *la* (12–14), (7–9) apart; setae *2a* (30–40), (22–26) apart; distance between setae *lb* and *la* (7–8), distance between setae *la* and *2a* (8–10). Setae *3a* (10–17), (9–10) apart. Opisthosoma with (68th–73th) dorsal annuli with minute conical microtubercles, pointed on the posterior annuli; (65) ventral annuli with pointed microtubercles. Setae *c2* (25–35), (45–52) apart, on (9th–10th) annulus; setae *d* (23–30), (24–30) apart, on (23rd–24th) annulus; setae *e* (11–13), (12–14) apart, on (37th–38th) annulus; setae *f* (22–25), (17–22) apart, on (60th–61st) annulus, (5th–6th) annulus from rear. Setae *h1* (3–4), (6) apart; setae *h2* (55–70), (9–10) apart; distance between *h1* and *h2* (2).

Type material—Holotype female and paratypes: 29 females, 11 males, 5 nymphs, collected from *Bromus inermis* (Leyss) (Poaceae) in Poznań, Poland (52.46 N, 16.93E). Leg. A. Skoracka, M. Hornyák. DNA barcode: 603-bp fragment of the cytochrome oxidase subunit I (COI) gene (Genbank Acc. No. MG267341), 509-bp fragment of the 28S rDNA D2 fragment (Genbank Acc. No. MG773120). Material is deposited in the collection of the Faculty of Biology, Adam Mickiewicz University in Poznań, Poland.

Etymology—*Plumiger*: feathered, covered with feathers, feather-bearing; the specific name (adjective in the nominative case, masculine) is derived from characteristic feather-like wax structures on both sides of the opisthosoma and on the lateral margins of the prodorsal shield in adult females (Fig. 4).

Relation to host—Mites are vagrants, living in the furrows of the upper leaf surface.

Differential diagnosis—The new species is similar to other grass-associated *Abacarus* species, with similarity of spindleform body, dorsomedian ridge on opisthosoma, triangular prodorsal shield with a pattern of the lack of median line and two parallel admedian lines present, and 8–9 empodial rays. The new species can be differentiated from its sister species *A. longilobus* (distance in mtDNA COI: mean $\pm$ SE = $6.9 \pm 1.1\%$; distance in 28S

rDNA D2: $1.8 \pm 0.6\%$) by the complete admedian lines (in *A. longilobus*, admedian lines are incomplete in rear half of prodorsal shield), longer *sc* setae (21–36 in *A. plumiger*, 8–12 in *A. longilobus*), and dorsal microtubercles present on the whole annuli, whereas in *A. longilobus* they are present only on ridges. In addition, both species differ in the shape of prodorsal shield lobe (it is triangular in its anterior part in *A. longilobus* and spike-like in *A. plumiger*). Suboral plate is semicircular and irregularly dashed in *A. longilobus*, whereas it is picked in *A. plumiger* with dashes parallel vertically. New species is also unique among other species from *Abacarus* complex by producing long feather-like wax structures at the margins of prodorsal shield. It also produces long wax filaments at the lateral parts of opisthosoma (Fig. 4a), much longer than *A. hystrix*, which also produces wax on its middle ridge (Skoracka 2009).

Discussion

Cryptic speciation is a common phenomenon in all mite taxa (Skoracka et al. 2015; Coimbra-Dores et al. 2016; García-Jiménez et al. 2017; de Rojas et al. 2018; Stork 2018), including obligatory phytophagous eriophyoid mites (Lewandowski et al. 2014; Cvrković et al. 2016). Our study also contributed to disclosing mites' cryptic diversity by demonstrating the existence of highly diverse lineages within the formerly described eriophyoid *A. hystrix* complex, that exhibit genetic variation along with variation in host use. Therefore, further consideration of *A. hystrix* status as a generalist species can be questioned. Additionally, the results presented here identified another member of the complex, *A. plumiger*, and at least seven additional genetic lineages within the complex that are putative separate species (*Abacarus* sp. 1–7; Fig. 2).

Members of the *Abacarus* genus are worldwide distributed and, so far, 65 species have been described. Among them, 37 species are specialized to monocotyledons, mainly grasses, and 29 to dicotyledonous plants (Amrine and Stasny 1994; J. Amrine unpublished eriophyoid database). Our analysis revealed that the genetic variation of grass-associated *Abacarus* members is much higher than anticipated, and suggests that the whole genus may need further integrative taxonomic research.

Accurate identification of cryptic species within eriophyoid mites is important especially in those taxa that are under scrutiny because of their virus transmission capacity, invasive potential and host range that involves economically important plants, such as, for example, cereals. Their proper identification helps to understand their potential environmental impact (Skoracka et al. 2012, 2014a, b, 2017; Miller et al. 2013) and mitochondrial COI DNA barcoding is a valuable tool assisting in such biodiversity and applied research (Hebert et al. 2003a, b). Previous barcoding studies on Eriophyoidea delineated the genetic distances in COI genes as good estimators of interspecific variation. For example, the K2P distance in COI gene between morphologically similar grass-associated *A. tosichella* (WCM) and Amaryllidaceae-associated *Aceria tulipae* (Keifer) was estimated to be ca. 14% (Skoracka et al. 2014a), whereas the distances among lineages of the WCM species complex were ca. 16% (Skoracka et al. 2014b; Szydło et al. 2015). The study investigating various coniferous-associated eriophyoid species of the *Trisetacus* genus revealed a similar range of interspecific variation at 17–20% uncorrected distance (Lewandowski et al. 2014). The extensive research on 73 species from all three eriophyoid families (Diptilomiopidae, Eriophyidae and Phytoptidae) demonstrated the COI genetic distance between species at the range 11.8–36.6% (Chetverikov

et al. 2015). Results obtained in this study showed that divergence between *Abacarus* lineages in COI gene ranged from 6.6 to 26.5% (Table 2), with the distance between the newly described species *A. plumiger* and *A. lolii*, *A. hystrix*, *A. acutatus* ranging from ca. 20 to 26%. Such a distance corresponds with interspecific genetic levels pointed out by the above-mentioned studies in eriophyoid mites and by studies on other mite taxa in the same gene, e.g., 7.3–18.3% K2P distance in an *Oligonychus* spider mite (Tetranychidae) (Matsuda et al. 2012), ca. 19% uncorrected distance in a *Panonychus* spider mite (Toda et al. 2000), ca. 11–27% K2P in some water mites (Hydrachnidia) (Stålstedt et al. 2013), ca. 14–27% K2P distance in astigmatid mites (Yang et al. 2011), and a mean 14.8% K2P distance between two *Glaucal* feather mite species (Analloidea) (Dabert et al. 2008).

In addition to genetic differentiation within the grass-associated *Abacarus* complex, the lineages within the complex differ in their host range width. Some of the *Abacarus* lineages in this study were specialized toward one or few closely related plant species (e.g., *A. plumiger*, *A. lolii*, *Abacarus* sp. 6, *Abacarus* sp. collected in Turkey), whereas others were associated with several host plant species (*A. acutatus*, *A. longilobus*). A similar situation, in which some genetic lineages within a species complex are specialists and others seem to be rather generalists, has already been noticed in eriophyoid mites, specifically in WCM (Skoracka et al. 2013). WCM is a grass-associated eriophyoid mite that was for a long time considered as a generalist species hosted by about 100 species of grasses, including cereals (Navia et al. 2013). This unusually wide host range of a mite that belongs to a taxon generally reported to be highly host-specialized, was then questioned (Skoracka et al. 2010; Navia et al. 2013). Recent studies revealed that WCM is in fact a species complex that consists of several genetic lineages (probably cryptic species). Indeed, some of the lineages seem to be specialized to one or few closely related host plant species, whereas several lineages turned out to be generalists. Overall, the WCM complex lineages differ in their host specificity, invasive potential and capacity to transmit viruses (Miller et al. 2012; Skoracka et al. 2012, 2013, 2014a, b, 2017; Kuczyński et al. 2016; Wosula et al. 2016). Analogously, we can suspect that such different host-use strategies (narrow and broad specialization) are present within grass-associated *Abacarus* complex. Such differential host specialization can be expected based on the variation in life-history strategies of host plant species. Some of the hosts are annual (e.g., wheat, *Triticosecale* Wittm. ex A. Camus) and others are perennial (e.g., *Elymus repens* (L.) Gould, *Bromus inermis* Leyss.) (Hubbard et al. 1992). The difference in life-history strategy between plant species can be crucial for the evolution of eriophyoid mites' host specificity. Annual grasses, such as cereals, which are harvested (semi-)annually, create less stable and less constant environments when compared to perennials. In consequence, mites which feed on annual grasses may be adapted to a broader host range, because in some seasons their annual and primary host is unavailable. This strategy might allow mites to find a suitable host through the whole year, without the necessity to produce an overwintering deutogynous stage, as is known for many eriophyoid species inhabiting deciduous plants (Manson and Oldfield 1996). In this way, annuality can be a driver for host generalization. Our results lend some support for this hypothesis: *A. hystrix* sensu lato (specifically *Abacarus* species collected in Turkey, *Abacarus* complex 1, and *Abacarus* complex 2; Fig. 1) feed on both short- and long-lived plants that give them opportunity for population persistence all over the year. The opposite strategy was observed in other lineages within the *Abacarus* complex (specifically *A. longilobus*, *A. plumiger*, *A. lolii* and *Abacarus* sp. 1–7; Fig. 1) which were associated exclusively with perennials. These species may be highly specific to their host due to its availability during the year.

In our study, dealing with diversity within the grass-infesting *Abacarus* species complex, we were able to distinguish several lineages within sampled populations. However, relationships between them remain unresolved. Future phylogenetic study should employ several more nuclear and mitochondrial markers, or preferably the whole genomes, and more extensive sampling within the genus complex to address the phylogenetic story of this taxon. Moreover, we recommend to include *Abacarus* species that are associated with dicotyledonous host plants to test the monophyly of the genus and relationships between *Abacarus* spp. hosted by mono- and dicotyledonous plants. Furthermore, the occurrence on the same host plant species (smooth brome) of two lineages in this study—*A. plumiger* and *Abacarus* sp. 7—warrants deeper investigation. According to the competitive exclusion principle, species competing for the same limited resource (host plant in this case) cannot coexist at constant population values (Hardin 1960). It is striking that such potentially close species (in terms of their genetic variation and ecology) inhabit the same environment, hence their phylogenetic relatedness, possibility of hybridization, and competitive behavior should be examined.

Scientists suggest that cryptic speciation is common among formerly described species and extensive sampling, using integrative approach, may help to reveal cryptic speciation and report biodiversity (Skoracka et al. 2015; Stork, 2018). Extensive sampling and examination of the *Abacarus* complex genetic diversity, as an effort of our study, pointed out nine new putative species, including the description of one of them—*Abacarus plumiger*—and set the path for further studies of *Abacarus* genus.

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Author contribution AS, AL, WS designed the study, MH, WS, AS collected the material, AS, WS, AL, KKI, AM, AL analyzed the data, all authors wrote the manuscript and accepted its final version.

Data availability The nucleotide sequences analyzed during the current study are available in GenBank repository (Accession Numbers MG267316–MG267374, MG773120).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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