Mesopolobus incultus auct. (Hymenoptera: Pteromalidae) contains two distinct species: *Mesopolobus incultus* (Walker, 1834) and *Mesopolobus amyntor* (Walker, 1845)

Marko Nieminen, Christer Hansson, Mari Kekkonen & Veli Vikberg

Nieminen, M., Hansson, C., Kekkonen, M. & Vikberg, V. 2018: *Mesopolobus incultus* auct. (Hymenoptera: Pteromalidae) contains two distinct species: *Mesopolobus incultus* (Walker, 1834) and *Mesopolobus amyntor* (Walker, 1845). — Entomol. Fennica 29: 175–184.

Mesopolobus incultus auct. is hypothesized to consist of two different species, *M. amyntor* (Walker) and *M. incultus* (Walker). This hypothesis is supported by molecular (cytochrome *c* oxidase subunit I, i.e. COI), morphological and biological data. *Mesopolobus amyntor* is a primary parasitoid of *Mecinus pascuorum* (Gyllenhal) and *M. labilis* (Herbst) (Coleoptera: Curculionidae) on *Plantago lanceolata*. *Mesopolobus incultus* is a primary parasitoid of *Protapion fulvipes* (Geoffroy) (Coleoptera: Apionidae) on *Trifolium repens* and *T. pratense,* and has also been inferred to act as a secondary parasitoid (hyperparasitoid) of *Spintherus dubius* (Nees) (Hymenoptera: Pteromalidae) or *Bruchophagus gibbus* (Boheman) (Hymenoptera: Eurytomidae). The results of this study lead to following nomenclatural changes: *M. amyntor* is removed from synonymy under *M. incultus*, and *Pteromalus urgo*, *P. belesis* and *P. berecynthos,* all described by Walker, are synonymized under *M. amyntor*. The species are diagnosed with characters illustrated.

M. Nieminen, Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, N1G 2W1, Canada; and Metapopulation Research Centre, Department of Biosciences, University of Helsinki, FI-00014 Helsinki, Finland; E-mail: marko.nieminen@faunatica.fi
C. Hansson, Biological Museum (Entomology), Lund University, Sölvegatan 37, SE-22362 Lund, Sweden; and Scientific Associate, Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom; E-mail: christer.hansson@biol.lu.se
M. Kekkonen, Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, N1G 2W1, Canada; and Finnish Museum of Natural History, Zoology unit, University of Helsinki, P.O. Box 17, FI-00014 Helsinki, Finland; E-mail: mari.kekkonen@helsinki.fi
V. Vikberg, Liinalammintie 11 as. 6, FI-14200 Turenki, Finland; E-mail: veli.vikberg@pp.inet.fi

Received 11 December 2017, accepted 31 January 2018

1. Introduction

It has been suspected in some previous studies that the parasitoid wasp *Mesopolobus incultus* (Walker, 1834) actually consists of more than one species (Graham 1957, von Rosen 1958, Baur *et al*. 2007). Moreover, Nieminen and Vikberg (2015) reported preliminary DNA barcoding results from specimens identified as *M. incultus* using the key in Graham (1969) and reared from both *Plantago lanceolata* L. and *Trifolium repens* L. (parasitizing species of Coleoptera: Curculionoidea) strongly indicating the existence of two distinct species.

Ecological aspects of the insect community of *P. lanceolata* have been intensively studied in Åland Islands (SW Finland) and Germany in recent decades, including metapopulation, community and metacommunity studies (e.g., Lei *et al*. 1997, Nieminen *et al*. 2004, van Nouhuys & Hanski 2005, Ojanen *et al*. 2013, Herbst *et al*. 2017, Nieminen & van Nouhuys 2017). Therefore, the precise interspecific relationships of each species of this community are of crucial importance for ecological implications. In the case of *M. incultus*, its ecological role in the community would be fundamentally different depending on its degree of host specificity: if the taxon developing in a host beetle inhabiting the spikes of *P. lanceolata* and the taxon developing in another host beetle in the inflorescences of *Trifolium* spp. are distinct species, there appears to be two possibly host-specific parasitoid species instead of one multi-host species.

Here, we report results on (1) the morphology of specimens and (2) the genetic differences based on DNA barcodes for *Mesopolobus* specimens reared from hosts feeding on *P. lanceolata*, *Trifolium pratense* L. and *T. repens* in Finland and Sweden, as well as (3) the descriptions of diagnostic characters of the type specimens.

2. Materials and methods

2.1. Acronyms

CMN: private collection of Marko Nieminen, Finland.
CMRC: collection of the Metapopulation Research Centre, University of Helsinki, Helsinki, Finland.
CVV: private collection of Veli Vikberg, Finland.
MZH: Zoological Museum, Finnish Museum of Natural History, Helsinki, Finland.
MZLU: Biological Museum (Entomology), Lund University, Lund, Sweden.
NHM: Natural History Museum, London, United Kingdom.

2.2. Morphology

The terminology used here follows Graham (1969) and Baur *et al*. (2007), with some terms included from Gibson (1997).

2.3. Molecular data

Ten Finnish specimens reared from *P. lanceolata* and *T. repens* and morphologically identified by Veli Vikberg as *M. incultus* were sampled for DNA barcoding as a part of Finnish DNA barcoding initiative (FinBOL). Barcode (the 648 bp region of the mitochondrial cytochrome *c* oxidase subunit I selected as the standard barcode for animals, Hebert *et al*. 2003a, b) sequences were produced at the Canadian Centre for DNA Barcoding (CCDB) following standard protocols (deWaard *et al*. 2008). All associated information and images were uploaded to BOLD (Ratnasingham & Hebert 2007).

In order to increase the number of sequenced specimens, public DNA barcodes identified as *M. incultus* were downloaded from the Public Data Portal on BOLD (in total three records collected as a part of School Malaise Trap Program, Steinke *et al*. 2017). In addition, all public members of the two BINs (i.e., putative species automatically delineated based on barcode sequences on BOLD, Ratnasingham & Hebert 2013) associated with *M. incultus* records were downloaded (in total four records from Germany and Switzerland). One additional private *M. incultus* sequence from Canada was provided by Paul Hebert.

All sequences were aligned in Clustal Omega v1.2.4 (Sievers *et al*. 2011, McWilliam *et al*. 2013, Li *et al*. 2015), employing default settings and subsequently inspected visually for stop codons and trimmed to 660 bp in MEGA v7.0.14

(Kumar *et al*. 2016). Sequences were then converted to phylip format in ALTER (Glez-Peña *et al*. 2010). Maximum likelihood gene trees were reconstructed in RAxML BlackBox (Stamatakis *et al*. 2008), employing gamma model of rate heterogeneity and visually inspected in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). All information related to BINs was acquired from the Barcode Index Number System via BIN Database on BOLD (accessed on May 31, 2017).

3. Results

3.1. Species treatments

Mesopolobus incultus (Walker) (Fig. 1c, e)

Platyterma incultum Walker, 1834:340. Lectotype male in NHM, examined.
Platyterma femorale Walker, 1834:341. Lectotype female in NHM, examined.
Amblymerus stupidus Walker, 1834:348–349. Lectotype female in NHM, examined.
Pteromalus leodocus Walker, 1839:237. Lectotype male in NHM, examined.
Pteromalus ergias Walker, 1839:238. Lectotype male in NHM, examined.
Pteromalus lissos Walker 1848:125, 196. Lectotype male in NHM, examined.
Pteromalus clavicornis Walker, 1874:318. Holotype female in NHM, examined.
Eutelus (*Amblymerus*) *crassicornis* Thomson, 1878:80. Lectotype female in MZLU, examined.
Platymesopus incultus (Walker), Graham (1957: 229).
Xenocrepis inculta (Walker), von Rosen (1958: 236).
Mesopolobus incultus (Walker), von Rosen (1960:26–28).

Material. **Finland** (all coordinates in EUREF-FIN). Specimens from Finland in CMN, CVV and MZLU. In total 136 ♀♀ 29 ♂♂.

Specimens reared from inflorescences of *T. pretense*. Al: Lemland, Järsö (66746–7:81091), sampled (no. 45) on 21.VII.2012, 2 ♀♀, MN leg.

Specimens reared from inflorescences of *T. repens*. MN leg.: 1 ♀, Al: Eckerö, Storby (66992:80874), sampled on 26.VII.2016; 2 ♀♀, Al: Hammarland, Strömma (67075:81014), sampled (no. 17) on 20.VII.2012; 1 ♀, Al: Jomala, Önningeby (66845–7:81117), sampled (no. 66) on 21.VII.2012; 4 ♀♀, Al: Lemland, Järsö (66746–7:81105–6), sampled (no. 39) on 20.VII.2012; 1 ♂, Al: Mariehamn, Klinten (66846:81074), sampled on 26.VII.2016. VV leg.: 28 ♀♀ 19 ♂♂, Ta: Janakkala, Kalpalinna, Tennis (67570:83696), sampled on 30.VI.2016 (10 ♀♀ 8 ♂♂) and 7.VII.2016 (18 ♀♀ 11 ♂♂); 3 ♀♀, Ta: Janakkala, Turenki, Ahilammi (67555:83720), sampled on 15.VII.2012, one female was included in the DNA barcoding.

Specimens swept from *T. repens*, all VV leg. 2 ♀♀, Ta: Janakkala, Kalpalinna, Turistirinne (6757:8369), 29. and 30.V.2016; 84 ♀♀ 9 ♂♂, Ta: Janakkala, Kalpalinna, Tennis (67570: 83696), 7.VI.2016 (8 ♀♀), 10.VI.2016 (4 ♀♀), 13.VI.2016 (22 ♀♀), 22.VI.2016 (12 ♀♀), 27.VI.2016 (11 ♀♀), 30.VI.2016 (14 ♀♀), 10.VII.2016 (2 ♀♀), 19.VII.2016 (2 ♀♀), 27.VII.2016 (4 ♀♀ 6 ♂♂), 30.VII.2016 (5 ♀♀ 3 ♂♂); 9 ♀♀, Ta: Janakkala, Turenki, Ahilammi (67555:83720), 10.VII.2012 (5 ♀♀), 26.VII.2012 (4 ♀♀).

Sweden (Skåne) (all coordinates in decimal degrees). All specimens from Sweden in MZLU. In total 123 ♀♀ 56 ♂♂.

Specimens ex *T. repens*, all J. Berger leg. 15 ♀♀ 1 ♂, Lyngby, 55.596533 13.339217, 10.VII.2014; 3 ♂♂, Södra Möinge, 55.863664 12.979254, 15.VII.2014; 8 ♀♀ 12 ♂♂, Gummastorp, 55.762638 13.607227, 15.VII. 2014; 4 ♀♀ 13 ♂♂, Stävie, 55.762569 13.069867, 1.VII.2014; 3 ♀♀ 9 ♂♂, Östra Ingelstad, 55.528028 14.09741, 11.VII.2014; 35 ♀♀, Gylle, 55.40893 13.199526, 10.VII.2014; 1 ♀ 4 ♂♂, Skarhult, 55.804891 13.372695, 15.VII.2014; 48 ♀♀ 14 ♂♂, Beddinge, 55.4 13.45, 10.VII.2014.

Specimens from fields of oilseed rape in water tray, all J. Berger leg. 3 ♀♀, Klågerup, 55.597823 13.259241, 19.V.2010; 2 ♀♀, Trulstorp, 56.5 12.983333, 10.VI.2010; 1 ♀, Lund, 55.7055 13.1870, 31.V.2010; 3 ♀♀, Alberta, 55.625044 13.319889, 15.VI.2010.

Diagnosis, based on 8 type specimens listed above. Forewing speculum on dorsal surface extending to 0.2–0.5× length of marginal vein (Fig.

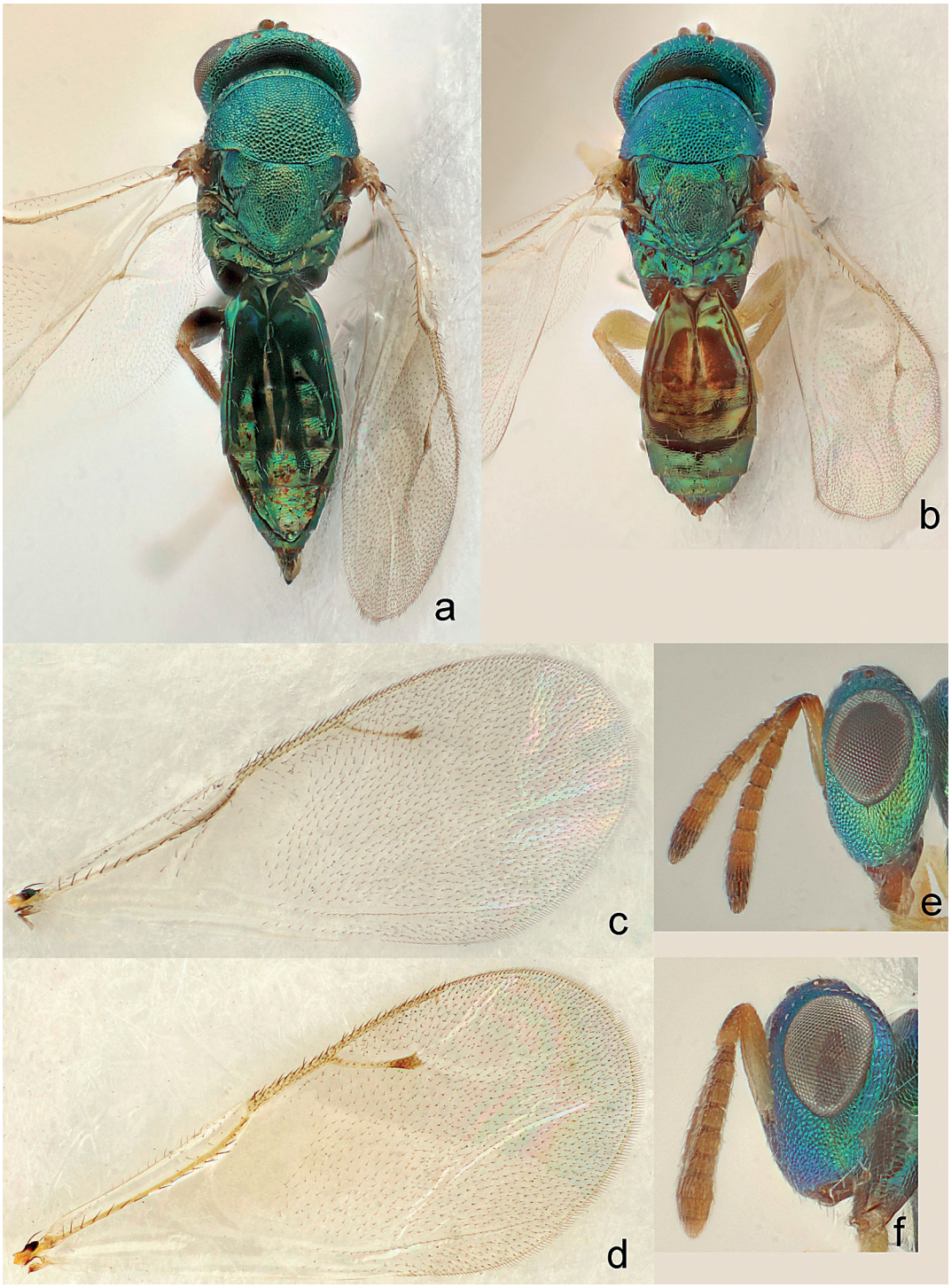

Fig. 1. *Mesopolobus* spp. All specimens are non-types from Finland, Al: Föglö, Överö, 66837:1395, 3.VIII.2000. – a. *M. amyntor* (Walker), female, habitus, dorsal aspect, length of specimen 2.1 mm. – b. *M. amyntor* male, habitus, dorsal aspect, length of specimen 1.8 mm. – c. *M. incultus* (Walker), female forewing. – d. *M. amyntor*, female forewing. – e. *M. incultus*, male head, lateral aspect. – f. *M. amyntor*, male head, lateral aspect.

1c); basal fold in female forewing with 1–7 setae (Fig. 1c); antennal clava in male blackish infuscate (Fig. 1e), i.e. distinctly darker than funiculars.

Descriptions. Female, based on a specimen reared from *T. repens* (Janakkala, Kalpalinna, collected 30.VI.2016, emerged 13.VII.2016; see above). Body 1.8 mm, forewing 1.5 mm. Tegula yellow. Clypeus reticulate, slightly striate, anteromedially smooth. Gaster length 1.00 mm, tip of hypopygium at 0.55 = 0.55× length of gaster; width 0.45 mm, length/width = 2.2×. Length of pedicel plus flagellum 0.50/head width 0.56 = 0.89×. Flagellum infuscate. Speculum on dorsal surface extending to 0.3× length of marginal vein, on ventral surface small setae extend to base of marginal vein. Basal fold with 2–3 setae, distal basal cell with 0–1 seta. Median area of propodeum with fine reticulate sculpture, plicae complete. Marginal vein 0.28/stigmal vein 0.17 = 1.65×. Mouth opening 0.20/malar space 0.15 = 1.33×. Mesosoma length 0.70 mm.

Male, based on a specimen reared from *T. repens* (Janakkala, Kalpalinna, collected 30.VI. 2016, emerged 13.VII.2016; see above). Body 1.6 mm, forewing 1.4 mm. Forewing marginal vein not inflated, 8.3× as long as broad. Median area of propodeum finely reticulate, plicae formed, but in anterior half indistinct. Head width 0.56/head height 0.47 = 1.19×. Head width/head length 0.27 = 2.07×, occiput rather strongly excavate. Tegula yellow. Gaster without pale spot medially. Clypeus reticulate, anteromedially with weak striae. Mouth opening 0.26/malar space 0.16 = 1.62×. Flagellum yellowish brown, clava almost entirely blackish infuscate. Marginal vein 50/stigmal vein 36 = 1.39×.

Variation in specimens from Finland, listed above. Females (124 specimens): Length of body 1.4–2.35 mm. Basal fold with 2–5 setae and apical basal cell with 0–3 setae, basal-most seta bordering speculum below basal 0.1–0.5 of marginal vein. Speculum reaching to basal 0.2–0.5 marginal vein. One swept large female (length of body 2.35 mm) with glabrous basal fold and apical part of basal cell, and with speculum extending to basal 0.3 of marginal vein.

Males (29 specimens): Length of body 0.75–1.55 mm.

Biology. Primary parasitoid of *Protapion fulvipes* (Geoffroy, 1785) (Coleoptera: Apionidae), which is oligophagous on *Trifolium*, on *T. repens* in Skåne, Sweden (von Rosen 1962). Some specimens were also reared from the inflorescences of *T. pratense* in Uppland, Sweden (von Rosen 1960), possibly as a secondary parasitoid (hyperparasitoid) of *Spintherus dubius* (Nees, 1834) (Hymenoptera: Pteromalidae) or *Bruchophagus gibbus* (Boheman, 1836) (Hymenoptera: Eurytomidae). However, this assumption on secondary parasitism was not confirmed by any means. Graham (1969) erroneously cited von Rosen (1962): "chiefly a hyperparasite (sometimes a primary parasite) of *Apion* spp. on *Trifolium repens* in Sweden". *Mesopolobus incultus* has often been reported as a hyperparasitoid in literature since that.

Mesopolobus amyntor (Walker), **stat. rev.** (Fig. 1a, b, d, f)

Pteromalus amyntor Walker, 1845:263. Lectotype female in NHM, examined.
Pteromalus urgo Walker, 1845:263. Lectotype female in NHM, examined. **syn. n**.
Pteromalus belesis Walker, 1848:125, 189. Lectotype male in NHM, examined. **syn. n**.
Pteromalus berecynthos Walker, 1848:125, 190. Lectotype male in NHM, examined. **syn. n**.

Material. **Finland** (all coordinates in EUREFFIN). Specimens in CMN, CVV, CMRC, MZH and MZLU. In total 20 ♀♀ 33 ♂♂.

Specimens reared from spikes of *P. lanceolate*, all MN leg. 4 ♀♀ 5 ♂♂, Al: Jomala, Önningeby, sampled (no. 62B [66842:81114], 67B [66846–7:81117], 67C, 71A [66853–4:81114], 71B & 71C) on 21.VII.2012; 10 ♀♀ 21 ♂♂, Al: Lemland, Järsö, sampled (no. 40A [66736–7:81105–6], 44B [66746–7:81091–2], 44E, 44F, 44H, 44i, 44J & 44K) on 21.VII.2012; 4 ♀♀ 6 ♂♂, Al: Lumparland, Svinö sampled (no. 48B [66788–9:81254], 48C, 48D & 48i) on 21.VII.2012; 2 ♀♀ 1 ♂, Al: Sund, Färjsundet, sampled (no. 33C [66983–4:81169–70] & 34A [66984–5:81139]) on 20.VII.2012.

Note. Body lengths (females 1.2–2.3 mm, males 0.7–1.9 mm) of specimens reared from *P. lanceolata* in Åland reported in Vikberg and

Table 1. Finnish specimens sampled for this study.

Orig. taxon	New taxon	BIN	ProcessID	Seq.	Prov.	Coll. date	Plant host	Insect host
Mi	*Ma*		FIMIS285-13	0	Sa	23.VII	*Pl*	*Mp*
Mi	*Mi*		FIMIS283-13	0	Ta	10.VII	*Tr*	unknown
Mi	*Mi*		FIMIS282-13	0	Ta	15.VII	*Tr*	unknown
Mi	*Ma*		FIMIS277-13	0	Al	20.VII	*Pl*	*Mp*
Mi	*Ma*		FIMIS278-13	228	Al	21.VII	*Pl*	*Mp*
Mi	*Ma*		FIMIS276-13	438	Al	20.VII	*Pl*	*Mp*
Mi	*Mi*	BOLD:ACJ6966	FIMIS284-13	592	Ta	26.VII	*Tr*	unknown
Mi	*Mi*	BOLD:ACJ6966	FIMIS281-13	635	Al	21.VII	*Tr*	unknown
Mi	*Mi*	BOLD:ACJ6966	FIMIS280-13	661	Al	21.VII	*Tr*	unknown
Mi	*Mi*	BOLD:ACJ6966	FIMIS279-13	661	Al	20.VII	*Tr*	unknown

Notes. Orig. taxon: Identification based on morphology (*Mi* = *Mesopolobus incultus*).
New taxon: New taxon name based on DNA barcode analysis, morphology and host plant (*Ma* = *Mesopolobus amyntor*).
BIN: Barcode Index Number identifier.
ProcessID: Process IDs of the records on BOLD.
Seq.: Length of DNA barcode sequence.
Prov.: Biogeographical province (Al = *Alandia*, Sa = *Savonia australis*, Ta = *Tavastia australis*).
Coll. date: Collection date in 2012.
Plant host: Plant species from which the sample specimen was reared (*Pl* = *Plantago lanceolata*; *Tr* = *Trifolium repens*).
Insect host: Insect species from which the sample specimen was reared (*Mp* = *Mecinus pascuorum*).
All voucher specimens stored at Friendship Park Research Centre, Finland.

Table 2. Specimens downloaded from the Public Data Portal of BOLD.

Original taxon (PDP)	Original taxon (BIN)	New taxon	Country	BIN	ProcessID	Inst.
M. incultus		*M. amyntor*	Canada		SMTPB14029-13	CBG
M. incultus		*M. amyntor*	Canada		SMTPB9284-13	CBG
M. incultus	*M. incultus*	*M. amyntor*	Canada	AAZ7491	SMTPI2547-14	CBG
Hymenoptera		*M. amyntor*	Canada		PHMTX866-11	CBG
Hymenoptera	Pteromalinae	*M. incultus*	Germany	ACJ6966	AMTPA1602-15	ZSM
Hymenoptera	Pteromalinae	*M. incultus*	Germany	ACJ6966	AMTPA2042-15	ZSM
Hymenoptera	Pteromalinae	*M. incultus*	Germany	ACJ6966	AMTPB2188-15	ZSM
Hymenoptera	*P. albipennis* group	*M. incultus*	Switzerland	ACJ6966	BCHYM12016-15	ZSM

Notes. Original taxon (PDP): Taxon name used in Public Data Portal of BOLD.
Original taxon (BIN): Taxon name on Public BIN page (*P. albipennis* group = *Pteromalus albipennis* group).
New taxon: New taxon name based on DNA barcode analysis, morphology and host plant.
BIN: Barcode Index Number identifier.
ProcessID: Process IDs of the records on BOLD.
Inst.: Institution storing the voucher specimen (CBG = University of Guelph, Centre for Biodiversity Genomics; ZSM = Staatliche naturwissenschaftliche Sammlungen Bayerns, Zoologische Staatssammlung Muenchen).

Nieminen (2012) were based on much larger material (2,148 ♀♀, 2,448 ♂♂).

Diagnosis, based on 4 type specimens listed above. Forewing speculum on dorsal surface extending to 0.6–1.0× length of marginal vein (Fig. 1d); basal fold in female glabrous, without setae (Fig. 1d); antennal clava in male yellowish brown (Fig. 1f), i.e. with same colour as funiculars.

Descriptions. Female (Fig. 1a), based on a specimen reared from *P. lanceolata* (Lemland, Järsö; sample no. 44B, see above). Body 2.2 mm, forewing 1.8 mm. Tegula yellow. Clypeus reticulate. Gaster length 1.17 mm, tip of hypopygium 0.74 = 0.63× length of gaster. Gaster: length 1.20/width 0.50 = 2.4×. Length of pedicel plus flagellum 0.57/head width 0.67 = 0.85×. Flagellum infuscate. Speculum on dorsal surface extending to 0.6× length of marginal vein, on ven-

Table 3. BINs associated with *Mesopolobus incultus* species complex.

BIN	Count	Countries	Mean	Max.	Dist.	Nearest BIN	Nearest member ID
BOLD:ACJ6966	8 (8)	FI, DE, CH	1.20	2.55	7.36	BOLD:ACL1509	Pteromalinae
BOLD:AAZ7491	32 (1)	CA, CH	0.65	3.32	3.55	BOLD:ABA8510	*M. incultus*

Notes. Count: Number of records in the BIN (number of public records in the BIN in p arentheses).
Countries: CA = Canada, CH = Switzerland, DE = Germany, FI = Finland.
Mean: Average intra-BIN distance (%).
Max.: Maximum intra-BIN distance (%);
Dist.: Distance to the nearest neighbour BIN in the BIN system (%).

tral surface with small setae below apical 0.6 of marginal vein. Basal fold and apical basal cell glabrous. Median area of propodeum almost smooth, anteriorly with short longitudinal folds, plicae formed but anteriorly weak. Marginal vein 0.32/stigmal vein 0.23 = 1.39×. Mouth opening 0.25/malar space 0.18 = 1.39×. Mesosoma length 0.85 mm.

Male (Fig. 1b), based on a specimen reared from *P. lanceolata* (Lemland, Järsö; sample no. 44B, see above). Body 1.85 mm, forewing 1.6 mm. Forewing marginal vein not inflated, 8.7× as long as broad. Median area of propodeum with very weak sculpture, plicae formed in posterior half. Head width 0.65/head height 0.55 = 1.18×. Head width/head length 0.30 = 2.2×, occiput rather strongly excavate. Tegula yellow. Gaster without pale spot medially. Clypeus reticulate, anteriorly with weak striae. Mouth opening 0.30/malar space 0.17 = 1.76×. Flagellum yellowish brown, slightly infuscate, clava not darker than funicular segments. Marginal vein 0.32/stigmal vein 0.23 = 1.39×.

Variation in specimens from Finland, listed above. Females (20 specimens): Length of body 1.35–2.25 mm (mean 1.88 mm). Basal fold with 0 setae (in one female 1 seta in one wing) and apical basal cell glabrous. Basalmost seta bordering speculum below apical 0.5–1.0 of marginal vein or speculum reaching to stigmal vein.

Males (34 specimens): Length of body 0.95–1.85 mm (mean 1.38 mm). Basal fold and apical part of basal cell together with 0–7 setae.

Biology. According to Graham (1969) probably a primary parasitoid of *Mecinus pascuorum* (Gyllenhal, 1813) (Coleoptera: Curculionidae) in seeds of *P. lanceolata*. This relationship has been confirmed in several other studies, as *M. incultus* has been reported being reared from the same host which is monophagous on *P. lanceolata* (e.g. Dickason 1968, Mohd Norowi *et al*. 2000, Hancock *et al*. 2013, Herbst *et al*. 2013, 2017, Wäschke *et al*. 2014, Nieminen & Vikberg 2015, Nieminen & van Nouhuys 2017). Moreover, Hancock *et al*. (2013), Herbst *et al*. (2013, 2017) and Wäschke *et al*. (2014) list another curculionid *Mecinus labilis* (Herbst, 1795), also monophagous on *P. lanceolata*, as a host of *M. incultus*.

3.2. Molecular data

Sequence data were obtained from six of ten Finnish samples (Table 1), so together with the eight public records (Table 2) the size of the molecular dataset was 14. The results of the molecular data supported the division of *M. incultus* into two species in concordance with morphology and plant species they were collected from. Four Finnish samples were associated with a BIN (BOLD:ACJ6966; Table 3). All these four specimens were reared from hosts on *T. repens*. Their host insect(s), however, remain unknown, as three potential host species emerged from the same collections (MN, unpublished data): *Hypera meles* (Fabricius, 1792) (Coleoptera: Curculionidae; polyphagous on Fabaceae), *P. fulvipes* and *Tychius picirostris* (Fabricius, 1787) (Coleoptera: Curculionidae; oligophagous on *Trifolium*). Four other members of the same BIN were collected from Germany or Switzerland and as they were public, they were included in analyses, but no host (insect or plant) information was available. These eight records formed a monophyletic, well-supported clade in ML tree (Fig. 2).

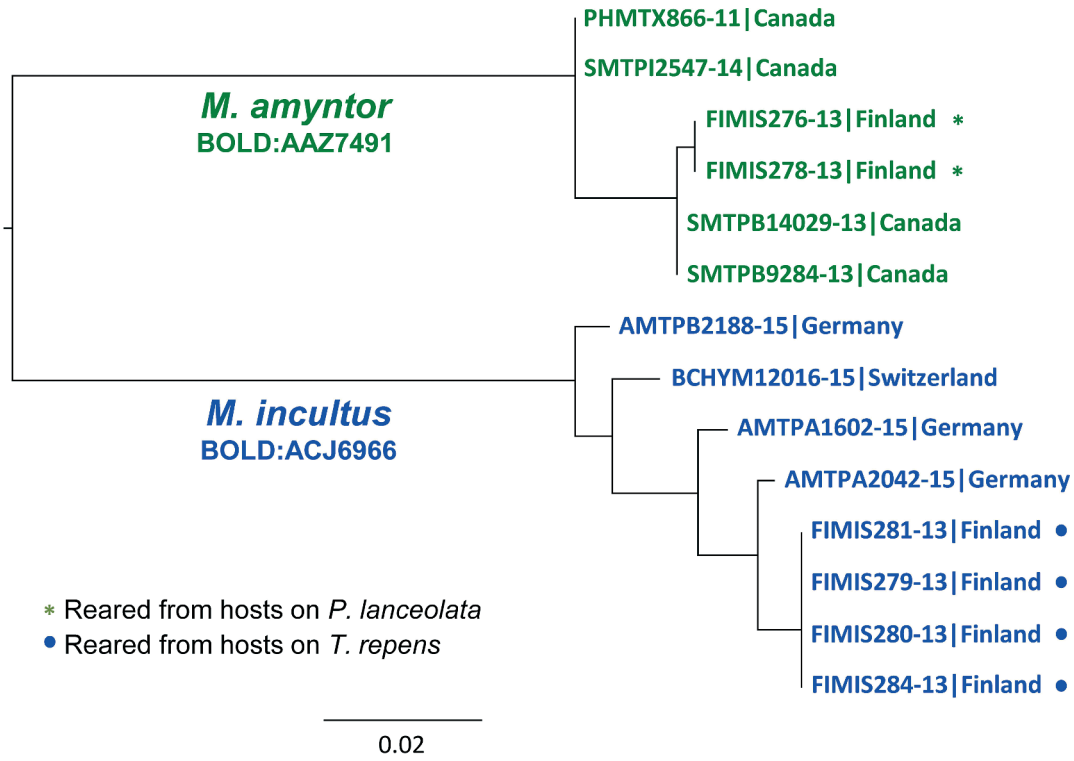

Fig. 2. Unrooted maximum likelihood gene tree based on the barcode region of mitochondrial COI. Bootstrap support value between the two clades is 100%. Tips show process IDs on BOLD (Ratnasingham & Hebert 2007), countries and host plant (if available) for each record.

The other two Finnish specimens with barcodes were not associated with any BIN on BOLD due to their short sequence length (438 and 228 bp). However, they formed a well-supported clade with the clade of the four Canadian specimens (Fig. 2) and hence can be associated with BIN BOLD:AAZ7491 (Table 3).

The Finnish members of this group were both reared from *P. lanceolata* and their insect host was *M. pascuorum*. Host insect and plant information was unavailable for the Canadian samples.

The two BINs associated with *M. incultus* were not each other's nearest neighbours in the BIN system on BOLD (Table 3). The nearest neighbour BIN of BOLD:ACJ6966 was BOLD: ACL1509, identified to subfamily level (Pteromalinae). This BIN included members from Bulgaria, Egypt, Saudi Arabia and South Africa. The nearest neighbour of BOLD: AAZ7491 included only one private member, which was also identified as *M. incultus.*

4. Discussion

All genetic and morphological evidence studied here are unequivocal: *Mesopolobus incultus* auct. contains two distinct species. Furthermore, the ecological interactions of these two parasitoid species are probably non-existent as they target different beetle hosts that live on different host plants. *Mesopolobus incultus* parasitizes at least the seed weevil *P. fulvipes* in the inflorescences of *T. pratense* and *T. repens*, and possibly also acts as a hyperparasitoid on other chalcidoid species, whereas *M. amyntor* apparently parasitizes the weevils *M. pascuorum* and *M. labilis* in the spikes of *P. lanceolata*.

However, the host spectra of both *Mesopolobus* species warrant further studies, as some relationships remain suggestive and yet others can be hypothesized. Nevertheless, the existence of two separate species instead of one elucidates community and metacommunity ecological studies of these sets of species by considerably reduc-

ing the number of actual and potential interactions.

Surprisingly, DNA barcodes suggest that the two species are not even close relatives as they are not each other's nearest neighbours in the BIN system, but phylogenetic analyses with enhanced sampling are needed to confirm this. It is also possible that a third species exists in this species complex because the nearest neighbour of BOLD:AAZ7491 was identified as *M. incultus*, but this cannot be studied here as only one private specimen belongs to this BIN. In conclusion, the results presented here demonstrate once more the power of integrative taxonomy, combining evidence from various sources for forming robust species hypotheses.

Acknowledgements. We thank Josef Berger (Biology Department, Lund University) for making available the material of Swedish *M. incultus* through MZLU, Natalie Dale-Skey for help with the type material of Walker types in NHM, Paul Hebert for providing a private *M. incultus* barcode sequence, Ilpo Mannerkoski for identifying some beetle material, Saskya van Nouhuys for comments on the manuscript and Gergely Várkonyi and the Finnish Barcode of Life project (FinBOL) for arranging the DNA barcoding of our samples.

References

Baur, H., Muller, F. J., Gibson, G. A. P., Mason, P. G. & Kuhlmann, U. 2007: A review of the species of *Mesopolobus* (Chalcidoidea: Pteromalidae) associated with *Ceutorhyncus* (Coleoptera: Curculionidae) host-species of European origin. — Bulletin of Entomological Research 97: 387–397.

deWaard, J. R., Ivanova, N. V., Hajibabaei, M. & Hebert, P. D. N. 2008: Assembling DNA Barcodes: Analytical Protocols. — In: Cristofre Martin, C. (ed.), Methods in Molecular Biology: Environmental Genetics: 275–293. Humana Press Inc., Totowa, USA. 364 pp.

Dickason, E. A. 1968: Observations on the biology of *Gymnaetron pascuorum* (Gyll.) (Coleoptera: Curculionidae). — The Coleopterists' Bulletin 22: 11–15.

Gibson, G. A. P. 1997: Morphology and Terminology. — In: Gibson, G. A. P., Huber, J. T. & Woolley, J. B. (eds.), Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera): 16–44. NRC Research press, Ottawa, Canada. 794 pp.

Glez-Peña, D., Gomez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F. & Posada, D. 2010: ALTER: program-oriented conversion of DNA and protein alignments. — Nucleic Acids Research 38: W14–W18.

Graham, M. W. R. de V. 1957: A revision of the Walker types of Pteromalidae (Hym., Chalcidoidea). Part 3 (including descriptions of new species). — Entomologist's Monthly Magazine 93: 217–236.

Graham, M. W. R. de V. 1969: The Pteromalidae of Northwestern Europe (Hymenoptera: Chalcidoidea). — Bulletin of the British Museum (Natural History) Entomology, Supplement 16: 1– 908.

Hancock, C., Wäschke, N., Schumacher, U., Linsenmair, K. E., Meiners, T. & Obermaier, E. 2013: Fertilizer application decreases insect abundance on *Plantago lanceolata*: a large-scale experiment in three geographic regions. — Arthropod–Plant Interactions 7: 147–158.

Hebert, P. D. N., Cywinska, A., Ball, S. & deWaard, J. 2003a: Biological identifications through DNA barcodes. — Proceedings of the Royal Society of London series B – Biological Sciences 270: 313–321.

Hebert, P. D. N., Ratnasingham, S. & deWaard, J. 2003b: Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. — Proceedings of the Royal Society of London series B – Biological Sciences 270 (Suppl 1): S96–S99.

Herting, B. 1973: Coleoptera to Strepsiptera. – A catalogue of parasites and predators of terrestrial arthropods. Section A. Host or Prey/Enemy. 3: 185 pp. Commonwealth Agricultural Bureaux, Institute of Biological Control.

Herbst, C., Arnold-Schwandner, S., Meiners, T., Peters, M. K., Rothenwöhrer, C., Steckel, J., Wäschke, N., Westphal, C. & Obermaier, E. 2017: Direct and indirect effects of agricultural intensification on a host-parasitoid system on the ribwort plantain (*Plantago lanceolata* L.) in a landscape context. — Landscape Ecology 32: 2015–2028.

Herbst, C., Wäschke, N., Barto, E. K., Arnold, S., Geuß, D., Halboth, I., Reschke, S., Sänger, J., Meiners, T. & Obermaier, E. 2013: Land use intensification in grasslands: higher trophic levels are more negatively affected than lower trophic levels. — Entomologia Experimentalis et Applicata 147: 269–281.

Kumar, S., Stecher, G. & Tamura, K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. — Molecular Biology and Evolution 33: 1870–1874.

Lei, G. C., Vikberg, V., Nieminen, M. & Kuussaari, M. 1997: The parasitoid complex attacking Finnish populations of the Glanville fritillary *Melitaea cinxia* (Lep: Nymphalidae), an endangered butterfly. — Journal of Natural History 31: 635–648.

Li, W., Cowley, A., Uludag, M., Gur, T., McWilliam, H., Squizzato, S., Park, Y. M., Buso, N. & Lopez, R. 2015: The EMBL-EBI bioinformatics web and programmatic tools framework. — Nucleic Acids Research 43(W1): W580–4.

McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., Cowley, A. P. & Lopez, R. 2013: Analysis Tool Web Services from the EMBL-EBI. — Nucleic Acids Research 41(Web Server issue): W597–600.

Mohd Norowi, H., Perry, J. N., Powell, W. & Rennolls, K. 2000: The effect of spatial scale on interactions be-

tween two weevils and their parasitoid. — Ecological Entomology 25: 188–196.

Nieminen, M. & van Nouhuys, S. 2017: The roles of trophic interactions, competition and landscape in determining metacommunity structure of a seed-feeding weevil and its parasitoids. — Annales Zoologici Fennici 54: 83–95.

Nieminen, M., Siljander, M. & Hanski, I. 2004: Structure and dynamics of *Melitaea cinxia* metapopulations. — In: Ehrlich, P. R. & Hanski, I. (eds.), On the Wings of Checkerspots: A model system for Population Biology: 63–91. Oxford University Press, Oxford. 408 pp.

Nieminen, M. & Vikberg, V. 2015: The insect community of *Plantago lanceolata* spikes in the Åland islands, SW Finland. — Entomologica Fennica 26: 30–52.

van Nouhuys, S. & Hanski, I. 2005: Metacommunities of butterflies, their host plants, and their parasitoids. — In: Holyoak, M., Leibold, M. A. & Holt, R. D. (eds.), Metacommunities – Spatial dynamics and ecological communities: 99–121. University of Chicago Press, London. 520 pp.

Ojanen, S., Nieminen, M., Meyke, E., Pöyry, J. & Hanski, I. 2013: Long-term metapopulation study of the Glanville fritillary butterfly (*Melitaea cinxia*): survey methods and data management. — Ecology and Evolution 3: 3713–3737.

Ratnasingham, S. & Hebert, P. D. N. 2007: BOLD: the Barcode of Life Data System (www.barcodinglife.org). — Molecular Ecology Notes 7: 355–364.

Ratnasingham, S. & Hebert, P. D. N. 2013: A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. — PLoS ONE 8: e66213.

von Rosen, H. 1958: Zur Kenntnis der europäischen Arten des Pteromaliden-Genus *Mesopolobus* Westwood 1833 (Hym., Chalc.). — Opuscula Entomologica 23: 203–240.

von Rosen, H. 1960: Zur Kenntnis des Pteromaliden-Genus *Mesopolobus* Westwood 1833 (Hym., Chalc.) VII. — Entomologisk Tidskrift 82: 1–48.

von Rosen, H. 1962: Zur Kenntnis des Pteromaliden-Genus *Mesopolobus* Westwood 1833 (Hym., Chalc.) IX. — Kungliga Lantbrukshögskolans Annaler 28: 141–148.

Sievers, F., Wilm, A., Dineen, D. G., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D. & Higgins, D. G. 2011: Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. — Molecular Systems Biology 7: 539.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008: A rapid bootstrap algorithm for the RAxML web-servers. — Systematic Biology 75: 758–771.

Steinke, D., Breton, V., Berzitis, E. & Hebert, P. D. N. 2017: The School Malaise Trap Program: coupling education outreach with scientific discovery. — PLoS Biology 15: e2001829.

Thomson, C. G. 1878: Hymenoptera Scandinaviae IV, *Pteromalus* (Swederus) continuatio. — Lund. 307 pp.

Vikberg, V. & Nieminen, M. 2012: *Mesopolobus incultus* (Walker) ja *Mesopolobus morys* (Walker) todettu Suomesta [*Mesopolobus incultus* (Walker) and *Mesopolobus morys* (Walker) found in Finland] (Hymenoptera: Chalcidoidea: Pteromalidae). — Sahlbergia 18: 8–13.

Walker, F. 1834: Monographia Chalciditum. — Entomological Magazine 2: 340–369.

Walker, F. 1839: Monographia Chalciditum. — Hyppolitys Baillière, Regent Street, London. 333 pp.

Walker, F. 1845: Characters of undescribed species of British Chalcidites. — Proceedings of the Linnean Society of London (Zoology) 1(24): 261–263.

Walker, F. 1848: List of the specimens of Hymenopterous insects in the collection of the British Museum, part 2, i–iv, 99–237. — E. Newman, London.

Walker, F. 1874: Descriptions of Amurland Chalcidiae. — Cistula Entomologica 1: 311–321.

Wäschke, N., Hardge, K., Hancock, C., Hilker, M., Obermaier, E. & Meiners, T. 2014: Habitats as complex odour environments: how does plant diversity affect herbivore and parasitoid orientation? — PloS ONE 9: e85152.