Several nominal species in one

An inventory of Norwegian species of *Pteromalus* (Hymenoptera: Pteromalidae) associated with Asteraceae plants.

Master of Science Thesis in Biodiversity and Systematics

Jon Peder Lindemann

NATURAL HISTORY MUSEUM
UNIVERSITY OF OSLO
2016

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http://www.duo.uio.no/

Trykk: Reprosentralen, Universitetet i Oslo

Preface

This master thesis was conducted at the Natural History Museum of Oslo in the period 2014-2016. It has given me the joy of studying biology from a comprehensive and exciting point of view. For this I want to thank my two supervisors, Arild Johnsen and Lars Ove Hansen. Thank you for giving me a great topic within the field of entomological systematics, and for your help and support during the last years! I also want to give a big thanks to Hannes Baur for his expertise and help, crucial for the identification of the *Pteromalus* material, and for his friendly hospitality during my visit in Bern. Furthermore, thanks to Gunnhild Marthinsen for the distribution of the insect material to Guelph, Jarl Andreas Anmarkrud for performing the lab work, and Karsten Sund for his nice photography's. You have been of invaluable importance to my master thesis! I also want to thank those who work at the Entomology Collection, including Geir Søli, Hallvard Elven, Dawn Williams and Trude Magnussen, for providing me with what I happened to need of necessary equipment, help and advices, and Leif Aarvik for identifying the moth, *Metzneria metzneriella*. Additionally, I want to thank the botanist Asbjørn Knutsen for giving me a private tour and lecture on Asteraceae plants at Bømlo. Finally, thanks to Helene Lind Jensen for proofreading, and my mother and father, family and friends for all your support.

Tøyen, 30 May 2016

Jon Peder Lindemann

Abstract

Frequently, DNA studies on parasitic Hymenoptera have revealed host specific cryptic species from complexes previously thought of as single generalist species. The parasitic Hymenoptera genus, *Pteromalus*, involves members that attack fruit flies (Diptera: Tephritidae) with variable degrees of host specificities, but with one species, *P. albipennis*, particularly standing out as a generalist. Many members of the genus are also very close morphologically, and the status of some of the species has therefore earlier been questioned. These trends indicate a potential presence of cryptic species, or the opposite, that several nominal species exist within the boundaries of a single species. To test these assumptions, species of *Pteromalus* were investigated based on morphological determinations, sequence data and their host fruit fly relations. The insects were hatched from different Asteraceae plants, and sequence analyses of the *Pteromalus* specimens were conducted based on two loci, the mitochondrial COI and nuclear ITS2 regions. Despite large intraspecific genetic variation in the two loci, no clear indication on cryptic species was revealed. This indicates that the observed polymorphism is caused by other factors, such as population size, speciation in reverse, Introgressive hybridization or *Wolbachia* infection. In two cases, sequence analyses were not able to distinguish between species of *Pteromalus*, suggesting that what is currently recognized as seven valid species rather exists within the boundaries of two. These results indicate that the two species, *P. intermedius* and *P. albipennis*, not constitute complexes of host specific cryptic species, but possess broader ranges of host fruit fly preferences than previously expected.

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Introduction

Taxonomic work on insects has been largely neglected (Wheeler 1990), despite that well above half of the currently described biodiversity is composed of insects (Grimaldi & Engel 2005). This is paradoxical both in an economical view and in the view of a scientist, as natural ecosystems increasingly have been transformed by humans during the last decades (Wheeler & Cracraft 1996, Berntsen & Hågvar 2010), potentially causing a mass extinction (Tilman *et al.* 1994, Wake & Vredenburg 2008). Today, biodiversity loss is considered to be one of the most serious global issues facing humanity (Thomas *et al.* 2004, Milennium Ecosystem Assessment 2005).

Because of this threat, the need to investigate biolodiversity faster is agreed upon and emphasized among biologists, but the way to do taxonomy more efficient and accurate has been under debate (Will & Rubinoff 2004, Will *et al.* 2005, DeSalle *et al.* 2005). The use of a single locus marker as a DNA barcode for all animals has been proposed as a promising solution (Hebert *et al.* 2003a, Savolainen *et al.* 2005). Several studies have shown that a 648bp region of the mitochondrial cytochrome c oxidase subunit 1 (COI), can be used to distinguish among closely related animal species (Hebert *et al.* 2003a, Hebert *et al.* 2003b, Hebert *et al.* 2004, Hajibabaei *et al.* 2005, Smith *et al.* 2005). Today, DNA barcoding has become an easy accessible tool, for species delimitation and identification. Expanding on this, an integrative taxonomy have been proposed, which uses a number of characters and traits, such as morphology, ecology and DNA, to accurately delimit species and taxa at different levels (Dayrat 2005, Will *et al.* 2005, Padial 2010, Schlick-Steiner 2010).

Great effort are being put into finding cryptic species (Bickford *et al.* 2007), i.e. two or more species that are, or have been, classified under the same nominal due to high morphological similarities (Bickford *et al.* 2007). Mayr (1963) theoretically facilitated for the increased focus on cryptic species when he introduced the biological species concept. Unlike the traditional morphological species concept (Mayden 1997), the biological species concept with a basic idea that speciation occurs when populations are isolated and gene flow between them has stopped (Mayr 1963, Smith 1966), are consistent with the idea that

species can be morphologically indistinguishable. Although cryptic species commonly is associated with the recent development of molecular species delimitation methods, which during the last decades truly have accelerated the rate of publications on this topic (Bickford *et al.* 2007), the concept has been used for a longer time (Janzon 1984). Compared to the many scientific papers on revealing cryptic animal species, very little is published on the opposite, that two or more nominal species exists within the boundaries of a single species. This may not only have to do with the taxonomical difficulties of many groups, but also the invested taxonomical effort (Bickford *et al.* 2007). E.g. recent molecular studies on molluscs, which traditionally have been extensively studied by "splitters", have resulted in several species being synonymized (Knowlton 2000, Prié & Puillandre 2014).

Cryptic species may be more common to occur in certain groups of species, where speciation is driven by mechanisms that do not alter morphological change (Bickford *et al.* 2007). Several studies on parasitic Hymenoptera have revealed complexes of host specific cryptic species, from species that previously were thought to be generalists (Kankare *et al.* 2005, Smith *et al.* 2008, Kaartinen *et al.* 2010, Zhang *et al.* 2011, Hambäck *et al.* 2013). A proposed explanation to this pattern is that sympatric speciation of parasitic Hymenoptera, caused by isolation onto separate host species, is driven by the change in chemoreceptors used to locate the hosts, and this does not necessarily cause any morphological changes (Bickford *et al.* 2007).

In this study, members of the genus *Pteromalus* Svederus, 1795 (Hymenoptera: Pteromalidae), a group of parasitic Hymenoptera, have been investigated together with their hosts in the fruit fly family (Diptera: Tephritidae). The north-west European species of *Pteromauls* are treated in detail by Graham (1969) under the genera, *Pteromalus* Svederus, 1795 and *Habrocytus* Thomson, 1878. The genus *Habrocytus* were later synonymized with *Pteromalus* (Bouček & Graham 1978). Graham (1969) did also arrange the genus into several sub-groups, including the *Pteromalus albipennis* group, which includes members entirely restricted to hosts of fruit flies, mainly associated with flower heads of Asteraceae plants (Gijswijt 1972, Janzon 1984, Graham & Gijswijt 1991, Gijswijt 1999, Polaszek *et al.* 2004). However, Graham provided no morphological delimitation of his sub-groups.

Janzon (1984) described another sub-group of the *P. albipennis* group, defined as "species with the row of hairs on lower surface of costal cell broken medially", and he assigned six new species to the group (Janzon 1980, 1983, 1984). Morphologically these are nearly indistinguishable as there are almost no qualitative characters, and the quantitative character ratios which are described as diagnostic, are highly overlapping (Graham 1969, Janzon 1984, Janzon 1986). According to Janzon (1984) the species, *P. leucanthemi* Janzon, 1980, *P. arnicae* Janzon, 1984, *P. pilosellae* Janzon, 1984 and *P. albipennis*, are very closely related and have not yet acquired many morphological characters. They are however isolated from each other in space and/or time, due to ecological differences, and he suggests they are sibling or cryptic species, experiencing high evolutionary rates. As far as known, members of this group generally possess quite narrow host ranges, except for *P. albipennis* which is known to attack a wide range of host fruit flies (Janzon 1984, Noyes 2016).

There are many unresolved problems in the taxonomy of Pteromalus (Janzon 1984, Baur 2002), just as stated by Graham (1969 p. 495): "An exhaustive study of *Habrocytus* (Syn. *Pteromalus*), perhaps the largest genus of Pteromalidae, would itself be almost the work of a lifetime". With the high morphological similarities among members of the *P. albipennis* group, the status of some of the species has earlier been questioned (Polaszek et al 2004, Baur 2002). For example the delimitation of *P. leucanthemi* from *P. albipennis* which largely is based on the host fruit fly specificities of *P. leucanthemi* (Polaszek 2004, Janzon 1986). Another example is *P. eudecipiens* Özdikmen, 2011 which seem to vary from *P. albipennis* only as an allometric effect (Baur 2002).

Despite the large size of this genus, and the well-known morphological difficulties that exists, species delimitation based on molecular methods has not yet been applied on the group. Additionally, an improved knowledge on parasitoid-host relations may be a key to better understand the taxonomy of the group. Based on earlier studies on parasitic Hymenoptera and their host relations (Bickford *et al.* 2007, Hambäck *et al.* 2013), one can suggest that cryptic species might be present in generalist species of *Pteromalus*. Finally, as COI sequence data for only eight different European *Pteromalus* species now are available in BOLD (Rathnasingham & Hebert 2007), it is also necessary to develop a barcode library for

this group. A well-developed barcode library of *Pteromalus* species can make it easier and faster to identify new species, and perhaps make it possible to work with this insect group for others than experts.

In this study, an inventory of the Norwegian *Pteromalus* fauna associated with Asteraceae plants has been carried out, and species boundaries have been examined based on morphology, molecular data, and host fruit fly relations. *Pteromalus* parasitoids and their host fruit flies have been hatched from different Asteraceae plants, and sequence data of two loci, the mitochondrial COI and the nuclear Internal Transcribed Spacer 2 (ITS2), have been obtained from different *Pteromalus* specimens.

The main aim was to investigate how well species delimitation based on molecular data fit morphological determinations of *Pteromalus* species. It was predicted that if the two delimitation approaches proved to be inconsistent, this would be due to (a) the existence of cryptic species, or the opposite, (b) that two or several nominal species exists within the boundaries of as single species. A final aim was to identify host fruit fly relations of the hatched *Pteromalus* species, possibly in the light of new knowledge from the molecular analysis. Additionally, the work was going to be a step in the development of a barcode inventory over the Norwegian species of the group, as a part of the Norwegian barcode library through the Norwegian Barcode of Life network (NorBOL).

Material and methods

Study organisms

Pteromalus Svederus, 1795 (Hymenoptera: Pteromalidae)

Members of *Pteromalus* (Figure 1 and 2) usually live through a quite similar life cycle as their hosts (Graham 1969, White 1988, Janzon 1984). The adult wasp oviposit the fruit fly in the 2nd or 3rd larval instar, develops as endoparasite, and hibernate as larva inside the pupa of the host, within the flower head (Janzon 1984). Other members of the genus develop as endoparasites in other species of Diptera, Lepidoptera, Coleoptera, solitary and social Aculeata, as hyperparasitoids on cocoons of members of the families Ichneumonidae and Brachonidae, or even predators of spider egg-sacs (Polaszek *et al.* 2004).

It is perhaps the most species rich genus in the family, with about 505 described species, of which 373 are recorded from Europe (Noyes 2016). There is little knowledge about the occurrence of this group in Norway where only 23 species are recorded (Artsdatabanken 2016), compared to neighbouring countries, e.g. Sweden where 78 species are recorded (Hedqvist 2003 , Mitroiu 2016, Noyes 2016, Dyntaxa 2015). This may be due to the fact that the genus is a taxonomic complicated group, and also that there has been few, if any, experts on the genus in Norway.

No study has yet been performed where *Pteromalus* is delimited based on phylogenetic principles, and despite the thoroughly work conducted by Graham (1969) and Bouček & Rasplus (1991), there are currently not recognized any synapomorphies for the genus. However, the genus can easily be recognized by a combination of characters (Graham 1969, Bouček & Rasplus 1991): clypeus striate, its anterior margin truncate or weakly to strongly emarginate, always without a median tooth; flagellum with 2 anelli and 6 funicular segments; clava in females symmetrical; prepectus with relatively small upper triangular area; paraspiracular sulci rather deep and usually with some transverse costulae.

Figure 1 Female of *Pteromalus albipennis* Walker, 1835. Photography by Karsten Sund, Natural History Museum of Oslo.

Figure 2 Female of *Pteromalus berylli* Walker, 1835. Photography by Karsten Sund, Natural History Museum of Oslo.

Fruit flies (Diptera: Tephritidae)

Fruit flies (Figure 3 and 4) are a family of phytophagous specialists that attack a broad range of plant families, usually exploiting the fruits or seeds, but some are also associated with stems, roots, or they live as miners in the leaves (Christenson & Foote 1960, White 1988, Redfern 1983). Of the about 4000 species and 300 genera currently described, nearly 800 species and 140 genera are Palearctic, and 58 species and 29 genera are recorded from Norway (Christenson & Foote 1960, White 1988, Korneyev 2015). A substantial part of the fruit flies, belonging to the subfamily Tephritinae, attack flower heads of plants in the family Asteraceae (Christenson & Foote 1960), in which they develop as larvae and sometimes form galls of different complexities (White 1988, Redfern 1983, Janzon 1984). North-west European species are mostly univoltine, the larvae develops for 20 to 40 days, pupate in their host, and will usually hibernate as pupae inside the flower heads (Janzon 1984, Redfern 1983, Zwölfer 1983). Species of the genus *Tephritis* Latreille, 1804 are however known to hibernate outside the flower heads as adults (Janzon 1984). Because fruit flies are able to do severe damage on fruits and seeds, many members of the group are economically important in agriculture, both as pests on fruit crops (Christenson & Foote 1960, White & Wang 1992, Drew & Hancock 1994, Drew *et al.* 2005) and as biological control agents of invasive weeds (Peschken & Harris 1975, Peschken 1979, Redfern 1983, Woodburn 1993).

Figure 3 Female of *Chaetorellia jaceae* (Robineau-Desvoidy, 1830). Photography by Karsten Sund, Natural History Museum of Oslo.

Figure 4 Male of Urophora jaceana (Hering, 1935). Photography by Karsten Sund, Natural History Museum of Oslo.

Collecting and hatching

Flower heads and stems were sampled from 39 species of Asteraceae common to Norway (Mossberg & Stenberg 2012). Many of these plants are typical weeds, growing on roadsides and meadows (Mossberg & Stenberg 2012), and some are common as pests in crops and pastures (Redfern 1983). The plant material was collected from March to the end of September, mainly during 2013 to 2015, at various sites in southern Norway, in agricultural areas around the Oslo Fjord (Figure 5ab) and in Grimstad (Figure 5a). Some alpine species of Asteraceae were also collected at higher elevations of Aurland in western Norway (Figure 5a). The specific collecting sites were chosen according to the sizes of the plant sub-populations. This because species diversity of higher trophic levels is known to be positively related to patch size (Eber 2001, van Nouhuys 2005), thus a larger plant sub-population is more likely to be inhabited by *Pteromalus* species. Accurate positions of the collecting sites are given in the Appendix 1.

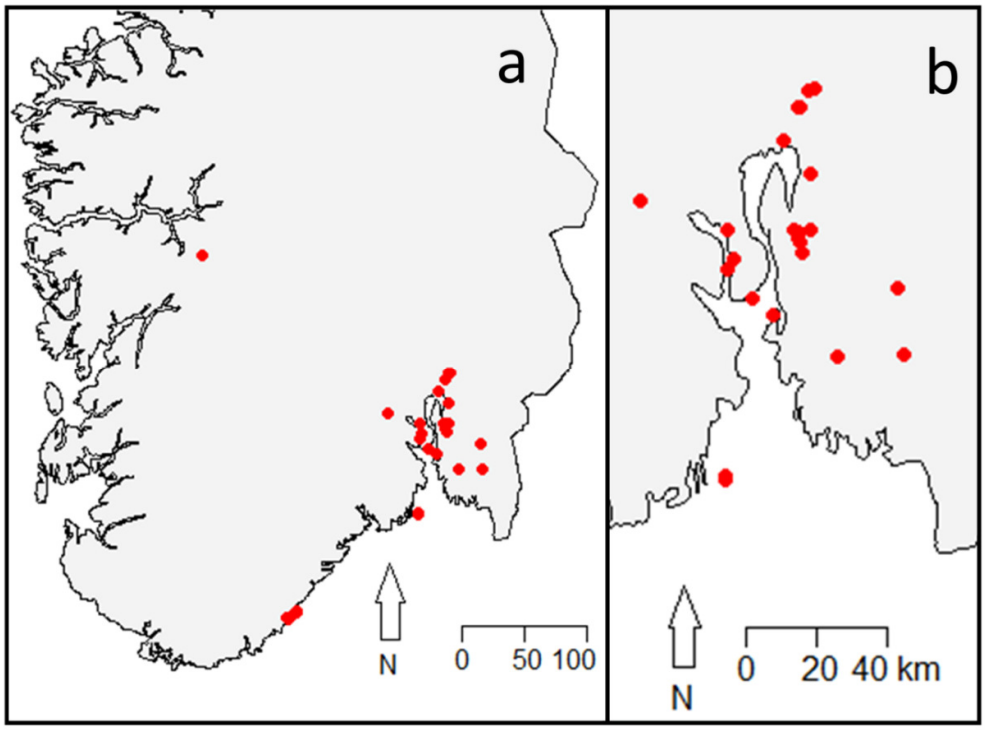

Figure 5 Map over Southern Norway **(a)** and the Oslo fjord region **(b)** with collecting sites marked with red dots. The collecting sites south-west of the Oslo Fjord that appear to lie out in the sea (b), are located at the island Tjøme.

Plant material was sorted according to collecting event, plant species and plant part, placed in specialized emergence boxes (Figure 6) and stored at temperatures between 20^{o}C and 25^{o}C for subsequent hatching of associated insects (Bakke 1955, Redfern 1983, Noyes 1982). Species of *Pteromalus* and fruit flies of the same hatching event, i.e. hatched from the same plant material within the same emergence box, were evaluated as possible parasitoid-host relations. Much of the plant material gathered in the spring was dead remains of last year's flowers, and the associated insects would then often emerge relatively quickly after the material was brought into the lab.

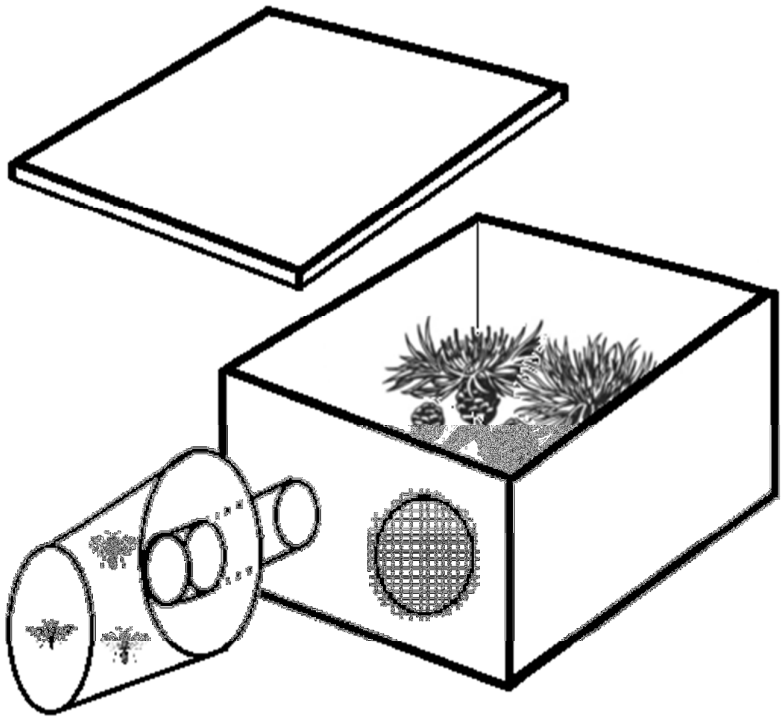

Figure 6 An emergence box consists of a dark storage room where the plant material is placed, an opening leading into a transparent "collecting house", and a hole in the box covered by net that release moisture to prevent mould and decay. As the insects hatch they will eventually fly towards the light and enter the collecting house. Del. J.P. Lindemann.

Preserving, mounting and identification

Reared insects were put to rest in a freezer and preserved in 96% ethanol in a refrigerator until they were mounted. To prevent the insect body parts from collapsing during drying from ethanol, the insect material was run through a drying process with hexamethyldisilazane (HMDS, Brown 1993, Quicke *et al.* 1999, Orozco & Gaimari 2016). *Pteromalus* specimens were card-mounted according to Noyes (1982, 2002), and the fruit flies were pinned with micro-pins according to White (1988).

A Leica M205C stereo microscope with a measure ocular was used for the species identification. *Pteromalus* specimens were identified to genus following the keys in Graham (1969) and Boucek & Rasplus (1991), and to species following Graham (1969) and Janzon (1984). In addition, *P. egregius* Förster, 1841 was treated in the study, a species that is morphologically very close to *P. albipennis*, and might just be a smaller form of the latter (Kurdjumov 1913). According to Kurdjumov (1913) *P. egregius* can be distinguished from the other by being slightly smaller and with a blue-greenish colour compared to bright blue in *P. albipennis*. One unidentified species was also treated, in this study addressed *Pteromalus sp.A.* Later the specimens were re-examined and identified by Hannes Baur, Naturhistorisches Museum der Burgergemeinde Bern.

The fruit flies were identified to species according to White (1988) and information provided on the Diptera.info web page (Diptera.info 2016). One unidentified fruit fly species of the genus *Campiglossa* was treated, addressed *Campiglossa sp.A*.

Material examined

The examined material of *Pteromalus* is presented in Appendix 1, where specimens can be identified by a four digit accession number (acc.no.). A total of 217 *Pteromalus* specimens and 430 fruit flies were treated in the study. Of *Pteromalus*, 156 specimens were of the recently reared material, and 61 specimens were selected from the NHM of Oslo collection. These museum objects have been collected or reared from various sites in southern Norway, and have previously been identified by Csaba Thuroczy. All the insect material used in this study is situated at The Natural History Museum of Oslo.

COI was first sampled from the 217 *Pteromalus* specimens, and ITS2 was later sampled from 32 specimens, selected according to the within species variation of COI (Table 4), and the topology of the COI gene tree (Figure 7). In this way the ITS2 region was used to check for congruence with COI, in species where COI showed high intraspecific variation. Species and number of individuals sampled for COI and ITS2 are presented in Table 1, and the specific specimens from which the loci were sampled are given in Appendix 1.

Males of *Pteromalus* are generally too variable to be determined by morphology, and were therefore mostly identified based on how they clustered with identified females in the sequence analysis. Sequences from 31 males were excluded because they could not be identified (Table 1).

Table 1 Number of specimens of the different species of *Pteromalus*, sampled for the COI and ITS loci. Some males could not be identified, neither on morphology, nor by sequence analysis as they didn't cluster with any identified females.

Pteromalus species	COI	ITS2
P. albipennis Walker, 1835	32	6
P. apum (Retzius, 1783)	5	4
P. arnicae Janzon, 1984	5	2
P. bedeguaris (Thomson, 1878)	1	
P. berylli Walker, 1835	14	
P. caudiger (Graham, 1969)	6	
P. chlorogaster (Thomson, 1878)	4	
P. chlorospilus (Walker, 1834)	2	
P. chrysos Walker, 1836	3	
P. cioni (Thomson, 1878)	3	
P. dispar (Curtis, 1827)	10	
P. egregius Förster, 1841	6	3
P. elevatus (Walker, 1834)	21	9
P. eudecipiens (Özdikman, 2011)	4	1
P. fasciatus (Thomson, 1878)	2	
P. hieracii (Thomsom, 1878)	4	
P. intermedius (Walker, 1834)	15	2
P. leucanthemi Janzon, 1980	6	1
P. musaeus Walker, 1844	3	3
P. patro Walker, 1848	3	
P. platyphilus Walker, 1874	2	
P. puparum (Linnaeus, 1758)	3	
P. rhinthon Walker, 1844	1	
P. semotus (Walker, 1834)	8	
P. sequester Walker, 1874	4	
P. sonchi Janzon, 1983	7	1
P. temporalis (Graham, 1969)	5	
P. tibiellus Zetterstedt, 1838	1	
P. sp.A	6	
Unidentified males	31	
Total	217	32

DNA extraction, amplification and sequencing

Mid legs of the examined *Pteromalus* specimens were sampled into 96-well plates with absolute ethanol, and sent to the Canadian Centre for DNA Barcoding (CCDB) in Guelph where COI sequence data were obtained. Here the DNA extraction, amplification and sequencing were performed following the CCDBs standard high-throughput protocols (Ivanova *et al.* 2006, deWaard *et al.* 2008, CCDB 2016). In the amplification of the 658bp region of COI, the forward primer LepF1 and reverse primer LepR1 (Hebert *et al.* 2004) were used. The COI sequence data are available in the project NOPRA at the BOLD Systems web platform (Ratnasingham & Hebert 2007).

ITS2 sequence data was obtained from fore legs by lab manager of the Natural History Museum of Oslo lab, Jarl Andreas Anmarkrud. DNA extraction was carried out with the Omega E.Z.N.A Tissue kit (Omega Bio-tek Inc, Norcross, GA) according to the manufacturer's tissue spin protocol. Before the tissue was lysed with TL-buffer and proteinase-K overnight, each insect leg was ground with two tungsten beads, with the purpose of making the tissue more accessible for the proteinase. The ITS2 region was amplified using the forward primer FFA (Brown *et al.* 2000) and reverse primer ITS4 (White *et al.* 1990), following a PCR protocol presented in Appendix 2. The PCR products were cleaned with Illustra ExoProStar (GE Helthcare Life Sciences) and sequenced in both directions on an ABI 3130 genetic analyser with BigDye v3.1 chemistry (Applied Biosystems) according to manufacturer's recommendations.

Raw sequence data (AB1 files) from the ITS2 sequencing were run in Codon Code Aligner v.2.4.7 (Codon Code Corporation) where the reads were assembled into contigs and the primers at the ends were cut off. The quality of the data was evaluated visually by inspecting the trace files, and seven samples were then excluded due to poor quality of the reads. All the contigs were manually inspected, and if necessary corrected, before the consensus sequences were ready.

Poor sequencing

The sampling of COI yielded relatively poor results, as only 129 sequences were obtained from the 217 specimens, corresponding to 59% success. It appeared that the sequence quality frequently dropped abruptly after a poly-T region. This is a known issue that unfolds during the PCR when different copies are generated that differs in length of the poly-T region, due to Taq Polymerase slippage, making the peaks after this region overlapping and unreadable (Clarke *et al.* 2001, Riepsamen *et al.* 2011, Zhou *et al.* 2013). However, the age was also an important factor as the museum objects, with an average age of 15.8 years, gave low success (52%) relative to the rest of the material (65%). The ITS2 results were better with 79% success, but also in this case the older specimens gave a much lower success.

Due to lack of sequence data, *P. bedeguaris, P. caudiger, P. chlorogaster, P. chrysos, P. fasciatus, P. hieracii, P. patro, P. platyphilus, P. puparum,* and *P. sequester* could not be included in the COI analysis, and *P. intermedius, P. sonchi and P. apum* could not be included the ITS2 analysis.

Data analysis

Sequence alignments were conducted in MEGA6 (Tamura *et al.* 2013) using the ClustalW algorithm (Thompson *et al.* 1994) with the default settings. Eleven sequences were excluded from the dataset because they were too short (<300 bp). Alignments were inspected visually for stop codons by translating the sequences into amino acid sequences in MEGA6. Genetic distances between and within species were calculated in MEGA6 with a K2P substitution model. Substitution models for each of the loci were evaluated with the Bayesian Information Criteria (BIC) (Schwarz 1978), in an automated model selection that was run in PAUP* v4.0a147 (Swafford 2002). This selected a HKY+G+I model for the CO1 alignment and a HKY+G model for the ITS2 alignment, which were used in the following sequence analyses.

Two Bayesian analyses were carried out in BEAST v1.8.1 (Drummond & Rambaut 2007, Drummond *et al.* 2012), one for each locus. These were run with a strict molecular clock, and a Yule speciation model was used as a tree prior to model the linage birth rate (Yule 1925; Gerhard 2008). The data was partitioned into codon positions 1+2 and 3, and the substitution rate, rate heterogeneity, and base frequencies were unlinked across codon positions. BEAST input files (XML files) were generated in BEAUTi v1.8.1 (Drummond *et al.* 2012), with the remaining priors left to their default values.

Another Bayesian analysis was carried out with both the loci included, using the multispecies coalescent model implemented in *BEAST (Heled & Drummond 2010), which estimates species tree topology from multiple loci sampled from multiple specimens. A normal *BEAST analysis require prior assumptions of species delimitation (Heled & Drummond 2010). Such priors are very strong, and may be disadvantageous if there are uncertainties regarding the assignment of individuals to species, by giving the analysis too little room to solve these problems (Jones *et al.* 2014). To make the analysis capable of delimitating species, it was performed with the DISSECT approach (Jones *et al.* 2014), which makes it possible to estimate a species tree in a Bayesian context without any prior assumptions on species delimitation. Most of the analysis was set in BEAUTi as for a normal *BEAST run, but with each specimen defined as its own species, because in the DISSECT approach, "species" as they appear in BEAUTi should be set to as small clusters as possible (Jones et al 2014). Both the loci were set to strict clocks, with the CO1 partition set to one and ITS2 estimated relative to this. Furthermore the XML document was edited by hand according to the supplements of Jones *et al.* (2014), where a birth-death model was replaced with a birth-death-collapse model, and an operator for the origin height was added.

For each of the three analyses, two chains were run for 50 000 000 Markov Chain Monte Carlo (MCMC) generations, sampling parameter values every 5000 generation, and combined in LogCombiner v1.8.1 (Drummond & Rambaut 2007) with a 10% burn-in. Convergence and ESS values of the runs were examined in Tracer v1.6 (Rambaut 2014), and sampled trees were summarized into maximum clade credibility trees with mean node

heights using Tree Annotator v1.7.1 (Drummond & Rambaut 2007). Furthermore the tree-files were visualized and edited in FigTree 1.4.2 (MPE 2016).

To objectively delimit the species based on COI and ITS2, a General Mixed Yule Coalescent model (GMYC, Pons *et al.* 2006, Fujisawa & Baraclough 2013) was used in order to set optimal divergence thresholds for the COI and ITS2 gene trees. The model sets a threshold value on an ultra-metric input tree by calculating the Maximum Likelihood solution for a point in time, located between the coalescence and the speciation process, and the estimated number of species equals the number of linages crossing this threshold (Fujisawa & Baraclough 2013). For each of the gene trees, single-threshold GMYC analyses were carried out in R (R core team 2015) using the SPLITS package (Ezard *et al.* 2009).

Results

Sequence analyses

Specifications for the sequences obtained from the two analysed loci are given in Table 2. The COI sequences were returned with lengths of 401 to 652 bp, which when aligned had 208 variable sites, of which 188 were parsimony informative, and a total overlap of 355 sites. ITS2 returned sequences with lengths of 465 to 591 bp, and the alignment had 137 variable sites, of which 56 were parsimony informative, and a total overlap of 530 sites. For both loci the majority of the variable and parsimony informative sites were located on 3rd codon positions (Table 2).

Table 2 Specifications for sequence data of two analysed loci, the mitochondrial COI and the nuclear ITS2, obtained from *Pteromalus* specimens. Given is the number of taxa and specimens from which sequences were obtained, sequence lengths in base pairs (Bp), and the number of variable sites (V) and parsimony informative sites (PI) for the three codon positions.

Locus	N Taxa	N Specimens	Bp	Codon	V	PI
COI	18	104	401-652	1	44	37
				2	5	2
				3	159	149
ITS2	7	24	465-591	1	38	16
				2	43	18
				3	56	22

Between-species variations in COI (Table 3) range from 0.01 to 0.18, with average 0.12 ± 2SD [0.05 - 0.18], and the smallest variations occurring between *P. sonchi* and *P. intermedius* (0.01), *P. sp.A* and *P. dispar* (0.02), *P. arnicae* and *P. albipennis* (0.03), *P. arnicae* and *P. leucanthemi* (0.03), and *P. arnicae* and *P. egregius* (0.03). Within-species variations (Table 4) range from 0 to 0.38, with average 0.016 ± 2SD [0-0.046], and the largest variations occurring within *P. egregius* (0.48), *P. albipennis* (0.39) and *P. musaeus* (0.033).

Table 3 Genetic distances between species of *Pteromalus*, measured in the mitochondrial COI region, with a Kimura 2 parameter substitution model. The average genetic distance between species is 0.12 ± 2SD [0.05-0.18].

Species	*P. alb*	*P. leu*	*P. eud*	*P. mus*	*P. apu*	*P. ber*	*P. chl*	*P. cio*	*P. dis*	*P. egr*	*P. arn*	*P.sp.A*	*P. ele*	*P. son*	*P. int*	*P. rhi*	*P. sem*
P. albipennis																	
P. leucanthemi	0.05																
P. eudecipiens	0.06	0.07															
P. musaeus	0.12	0.12	0.11														
P. apum	0.12	0.13	0.12	0.11													
P. berylli	0.14	0.13	0.15	0.16	0.14												
P. chlorospilus	0.10	0.10	0.09	0.10	0.11	0.14											
P. cioni	0.13	0.14	0.10	0.13	0.14	0.16	0.11										
P. dispar	0.14	0.15	0.14	0.10	0.09	0.17	0.10	0.13									
P. egregius	0.04	0.03	0.06	0.12	0.12	0.13	0.10	0.13	0.15								
P. arnicae	0.03	0.03	0.05	0.12	0.12	0.14	0.09	0.12	0.15	0.03							
P. sp.A	0.15	0.16	0.15	0.10	0.10	0.17	0.11	0.14	0.02	0.15	0.15						
P. elevatus	0.13	0.13	0.13	0.10	0.13	0.15	0.11	0.13	0.14	0.13	0.13	0.14					
P. sonchi	0.14	0.14	0.13	0.10	0.11	0.16	0.07	0.12	0.10	0.14	0.14	0.10	0.11				
P. intermedius	0.14	0.14	0.13	0.10	0.11	0.16	0.07	0.12	0.10	0.14	0.14	0.10	0.11	0.01			
P. rhinthon	0.11	0.11	0.12	0.11	0.10	0.16	0.06	0.15	0.10	0.12	0.11	0.09	0.12	0.09	0.10		
P. semotus	0.12	0.12	0.12	0.08	0.10	0.13	0.08	0.11	0.09	0.12	0.12	0.10	0.11	0.07	0.08	0.11	
P. temporalis	0.11	0.12	0.10	0.13	0.12	0.18	0.09	0.14	0.16	0.11	0.11	0.17	0.15	0.13	0.13	0.12	0.12

Table 4 Genetic distances within species of *Pteromalus*, measured in the mitochondrial COI region, with a Kimura 2 parameter substitution model. The average genetic distance within species is 0.16 ± 2SD [0 - 0.046]. Four species are not applicable (n/a) for this analysis as they each are only represented with one specimen.

Species	Distances
P. egregius	0.048
P. albipennis	0.039
P. musaeus	0.033
P. elevatus	0.028
P. apum	0.024
P. cioni	0.011
P. berylli	0.010
P. intermedius	0.009
P. dispar	0.007
P. sp.A	0.005
P. sonchi	0.005
P. arnicae	0.000
P. semotus	0.002
P. temporalis	0
P. chlorospilus	NA
P. eudecipiens	NA
P. leucanthemi	NA
P. rhinthon	NA

The Bayesian analyses of COI and ITS2 (Figure 7) clustered most of the species in reciprocally monophyletic clades, supported by high posterior probabilities (>0.99). They were however not able to distinguish among *P. albipennis, P. arnicae , P. egregius*, *P. eudecipiens* and *P. leucanthem*i, which were intermixed in one clade (The *P. albipennis* clade). They were also not able to distinguish between *P. intermedius* and *P. sonchi* which were intermixed in another clade (The *P. intermedius* clade). Both the *P. albipennis* and *P. intermedius* clades were reciprocally monophyletic and supported by high posterior probabilities (>0.99). Internal nodes of the *P. albipennis* clade were separated by large genetic distances and supported by high posterior probabilities in both the CO1 and ITS2 trees, but these topologies were completely incongruent. Similarly, the *P. elevatus* clade in the COI tree had internal nodes separated by large genetic distances and supported by high posterior probabilities, but both distances and supports were much smaller in the ITS2 tree, and the topologies were incongruent. The two gene trees are congruent for the *P. albipennis* clade, *P. elevatus*, *P. musaeus* and the internal topology of *P. musaeus*.

The divergence threshold for the COI tree (Figure 8a) set by the general mixed yule coalescent model (GMYC), suggested 21 entities (putative species), 17 of which were clusters. The model matched the species, *P. cioni, P. semotus, P. sp.A, P. chlorospilus*, *P. temporalis* and *P. rhinton*. It assigned two entities to each of the species, *P. berylli, P. musaeus*, *P. intermedius* and *P. apum*. Three clusters were assigned to *P. elevatus*, and four clusters and one singleton to the *P. albipennis* clade. The GMYC threshold for the ITS2 tree suggested ten entities, four of which were clusters, matching completely *P. elevatus*, dividing *P. musaeus* into two, and *P. albipennis* into six entities (Figure 8b).

Neither the Bayesian analysis conducted under the multispecies coalescent (Figure 9) were able distinguished among the species in the *P. albipennis* clade, but unlike the previous analyses, this gave only low posterior probability supports (<0.6) to the internal nodes of the *P. albipennis* clade. Similarly low supports (<0.32) were given to the internal nodes of *P. elevatus*. Just as in the other analyses, *P. elevatus, P. musaeus* and the *P. albipennis* clade were well supported (>0.99), and the internal node of *P. musaeus* were given some support (0.78).

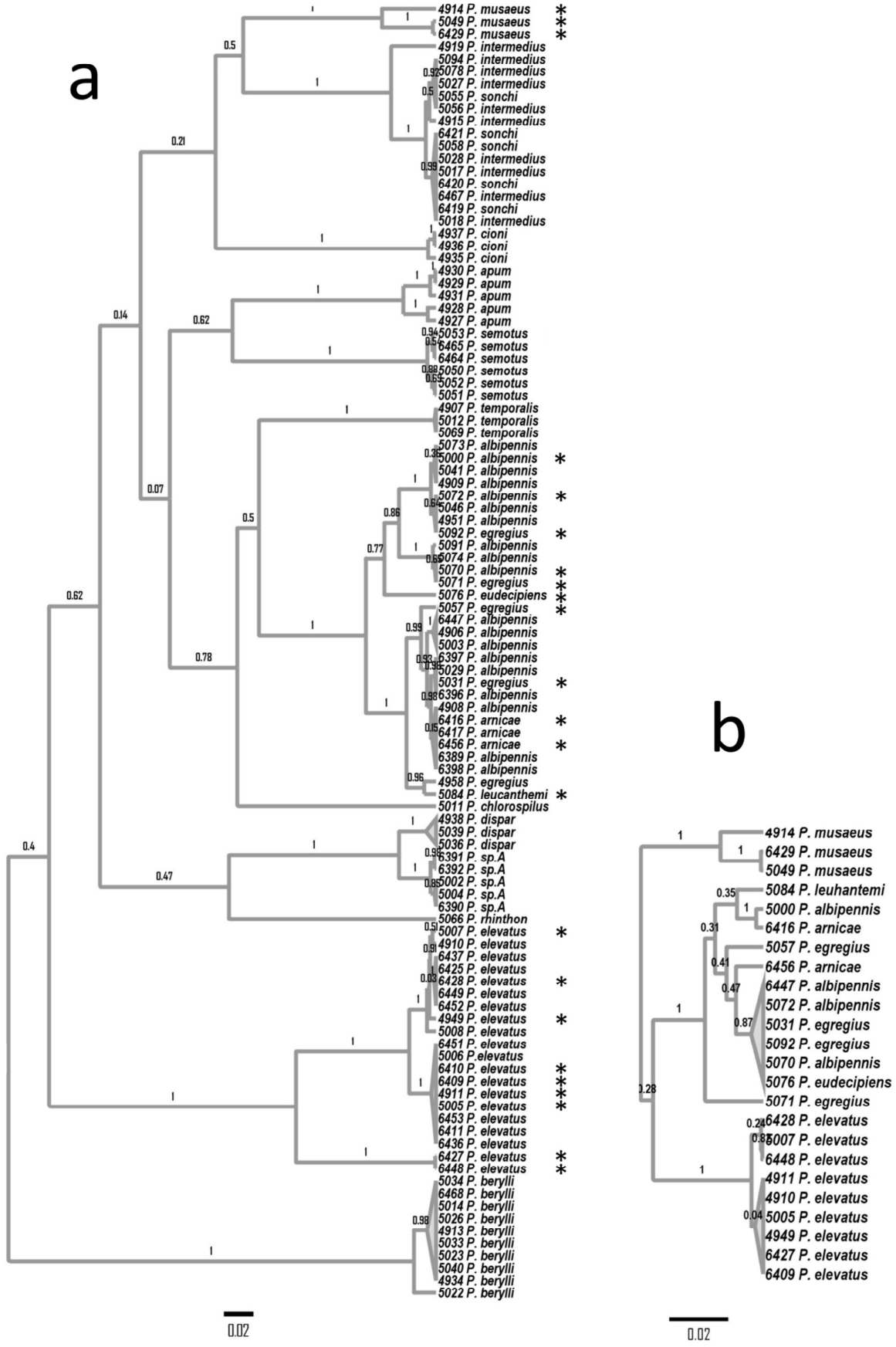

Figure 7 Maximum clade credibility trees from Bayesian analyses of the mitochondrial COI **(a)** and the nuclear ITS2 **(b)** regions. Posterior probability supports are labelled above each branch. Tips represent specimens identified by a four digit accession number. On the COI tree, tips corresponding to specimens included in the ITS2 tree are marked with an asterisk (*). Note that the COI gene tree is unable to distinguish *P. intermedius* from *P. sonchi*, and neither the COI nor the ITS2 gene trees are able to distinguish among *P. albipennis, P. arnicae, P. egregius*, *P. eudecipiens* or *P. leucanthemi*.

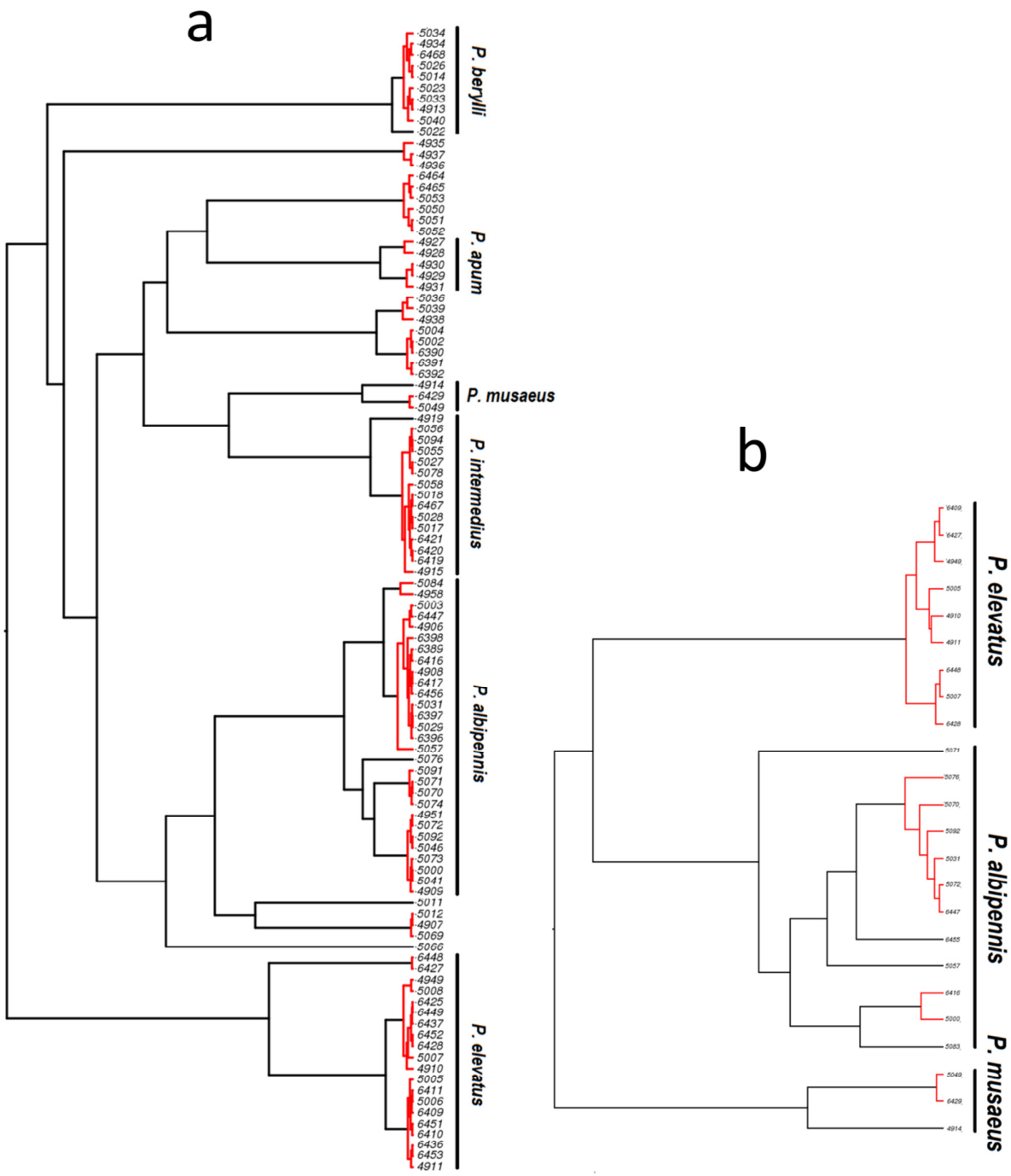

Figure 8 Output from species delimitation analyses based on the General Mixed Yule Coalescent model, with Bayesian maximum credibility gene trees of the mitochondrial COI **(a)** and nuclear ITS2 **(b)** regions, used as input trees. The Red branches indicate clusters recognized as species by the analysis. Taxa delimited into more than one entity are labelled. Tips represent specimens identified by a four digit accession number.

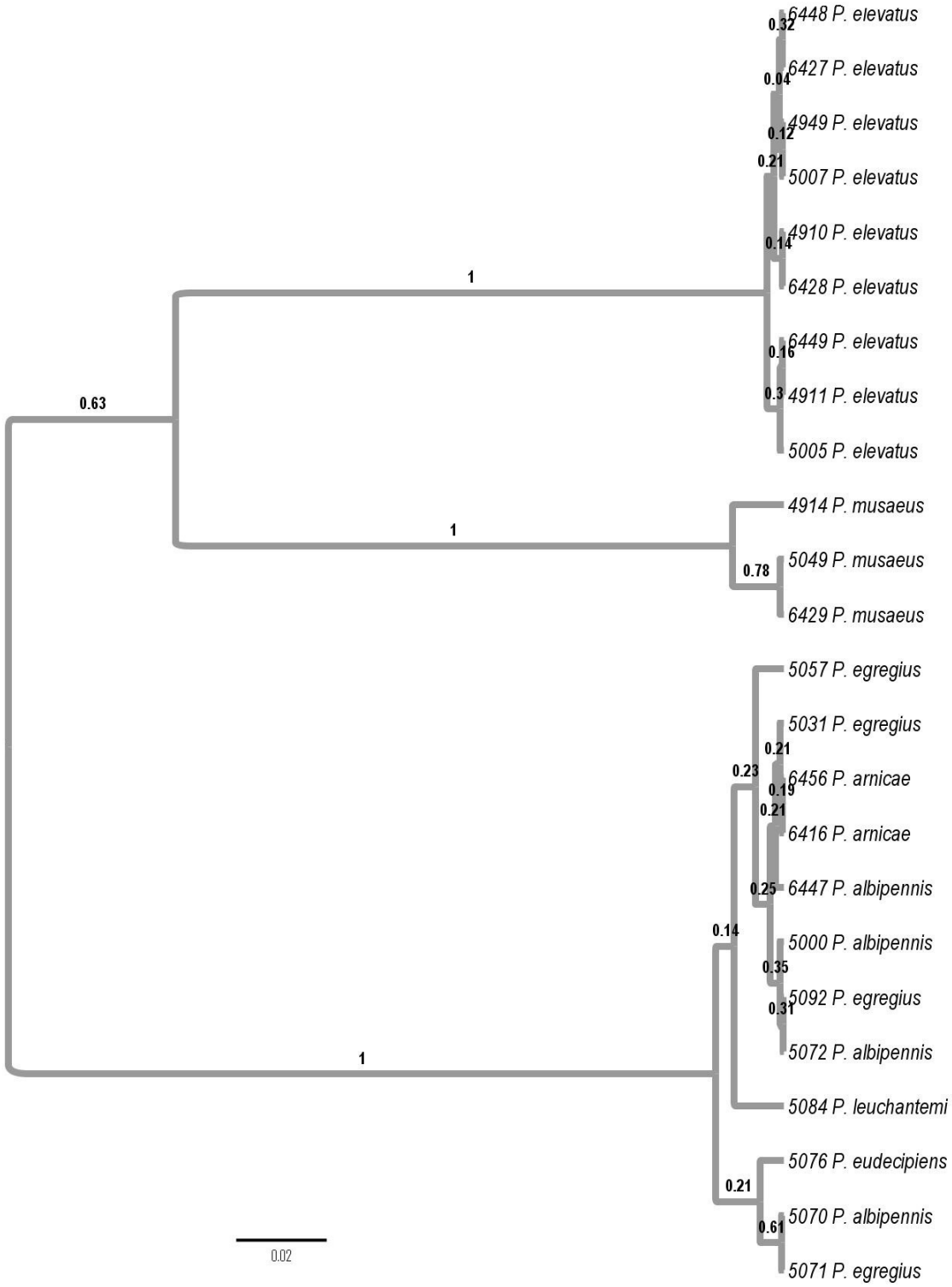

Figure 9 Maximum clade credibility tree from a Bayesian analysis based on the mitochondrial CO1 and the nuclear ITS2 regions, conducted under the multispecies coalescent model implemented in *BEAST, with the DISSECT approach. Posterior probability supports are labelled above each branch. Tips represent specimens identified by a four digit accession number. Note the low support of the internal nodes in the *P. albipennis* clade.

Hatching results

Out of the 39 sampled plant species, 22 species yielded specimens of *Pteromalus* and/or fruit flies from flower heads, and two species from stems. Species of *Pteromalus* and fruit flies emerging from the different plant species at separate hatching events are presented in Table 5. Plant species that were not recorded to yield any specimens of *Pteromalus* or fruit flies are given in Appendix 4.

A total of 22 fruit fly species were hatched, of which three did not emerge together with any *Pteromalus* parasitoids. These were *Oxyna parietina, Tephritis angustipennis* and *Xyphosa miliaria.* The species, *Chaetorellia jaceae* emerged together with either *P. semotus* or *P. chlorospilus* at three separate hatching events, but in all cases also together with other fruit fly species, which made it impossible to make any inference on parasitoid relations.

A total of 19 *Pteromalus* species were hatched, of which two, *P. caudiger* and *P. dispar,* did not emerge together with any host fruit flies. The species *P. semotus* emerged from *Centaurea nigra* together with the fruit fly species, *Chaetorellia jaceae* and *Chaetostomella cylindrica*, and the moth *Metzneria metzneriella* (Stainton, 1851) (Lepidoptera: Gelechiidae). Because this species earlier are recorded from a large number of host species, mainly in the Coleoptera and Lepidoptera, including members of the Gelichiidae (Noyes 2016, Graham 1969), it is most likely associated with the moth. The species, *P. hieracii,* emerged from both stems and flower heads of *Centaurea jacea*, in both cases together with gall wasps (Hymenoptera: Cynipidae).

Relations between species of the three trophic levels, *Pteromalus* parasitoids, host fruit flies and host plants are presented in figure 10. The different species in the *P. albipennis* clade are here treated as *P. albipennis*, and the species in the *P. intermedius* clade are treated as *P. intermedius.*

Table 5 *Pteromalus* and fruit fly species hatched from flower heads (FH) and stems (S) of different Norwegian Asteraceae plants. Each row describes a unique combination of species, and at how many separate hatching events (N) these emerged. Where no *Pteromalus* or fruit fly specimens emerged, the fields are marked not applicable (NA).

N	Plant species	Part	Fruit fly species	*Pteromalus* species
1	*Achillea ptarmica* L.	FH	*Tephritis angustipennis* (Loew, 1857)	NA
3	*Arctium tomentosum* Mill.	FH	*Tephritis bardanae* (Schrank, 1803)	*albipennis* Walker, 1835
2	*Arnica montana* L.	FH	*Tephritis arnicae* (Linnaeus, 1758)	arnicae Janzon, 1984
1	*Artemisia vulgaris* L.	S	*Oxyna parietina* (Linnaeus, 1758)	NA
1	*Carduus crispus* L.	FH	*Tephritis hyoscyami* (Linnaeus, 1758)	*temporalis* (Graham, 1969) *albipennis* Janzon, 1984
1	*Centaurea jacea* L.	FH	NA	*albipennis* Walker, 1835
2	*Centaurea jacea* L.	FH	*Urophora jaceana* (Hering, 1935)	*elevatus* (Walker, 1834)
1	*Centaurea jacea* L.	FH	*Chaetorellia jaceae* (Robineau-Desvoidy, 1830) *Urophora jaceana* (Hering, 1935)	*chlorospilus* (Walker, 1834)
1	*Centaurea jacea* L.	FH	NA	*hieracii* (Thomson, 1878)
1	*Centaurea jacea* L.	S	NA	*hieracii* (Thomson, 1878)
2	*Centaurea nigra* L.	FH	*Chaetorellia jaceae* (Robineau-Desvoidy, 1830) *Chaetostomella cylindrica* (Robineau-Desvoidy, 1830)	semotus (Walker, 1834)
2	*Centaurea scabiosa* L.	FH	*Urophora stylata* (Fabricius, 1755)	*elevatus* (Walker, 1834)
2	*Cicerbita alpina* Wallr.	FH	*Campiglossa guttella* (Rondani, 1870)	sp. A *albipennis* Walker, 1835
1	*Cirsium arvense* L.	FH	*Xyphosia miliaria* (Schrank, 1781)	NA
1	*Cirsium arvense* L.	FH	NA	*intermedius* (Walker, 1834)
1	*Cirsium arvense* L.	FH	NA	*sp. A*
1	*Cirsium arvense* L.	FH	*Tephritis cometa* (Loew, 1840)	*temporalis* (Graham, 1969)
1	*Cirsium arvense* L.	FH	*Tephritis cometa* (Loew, 1840)	*albipennis Walker, 1835* *egregius* Förster, 1841
1	*Cirsium arvense* L.	FH	*Tephritis cometa* (Loew, 1840)	*albipennis* Walker, 1835
1	*Cirsium heterophyllum* (L.) Hill.	FH	NA	*caudiger* (Graham, 1969)
1	*Cirsium heterophyllum* (L.) Hill.	FH	*Tephritis conura* (Loew, 1844)	*berylli* Walker, 1835
1	*Cirsium palustre* (L.) Scop.	FH	*Chaetostomella cylindrica* (Robineau-Desvoidy, 1830)	*berylli* Walker, 1835
1	*Cirsium palustre* (L.) Scop.	FH	NA	*dispar* (Curtis, 1827)
2	*Cirsium vulgare* (Savi) Ten.	FH	*Terellia serratulae* (Linnaeus, 1758)	*berylli* Walker, 1835 *albipennis* Walker, 1835
2	*Crepis paludosa* (L.) Moench	FH	*Campiglossa producta* (Loew, 1844)	*intermedius* (Walker, 1834) *albipennis* Walker, 1835
1	*Hieracium umbellatum* L.	FH	NA	*albipennis* Walker, 1835
1	*Hieracium umbellatum* L.	FH	*Noeeta pupillata* (Fallen, 1814)	*musaeus* Walker, 1844
1	*Hypochaeris radicata* L.	FH	Tephritis vespertina (Loew, 1844)	albipennis Walker, 1835
1	*Hypochaeris radicata* L.	FH	*Tephritis vespertina* (Loew, 1844)	*intermedius* (Walker, 1834)
1	*Leucanthemum vulgare* Lam.	FH	*Tephritis neesii* (Meigen, 1830)	*leucanthemi* Janzon, 1980
1	*Pilosella lactucella* Wallr.	FH	NA	*musaeus* Walker, 1844
2	*Pilosella peletariana* (Mérat) F.W.S. &	FH	*Tephritis ruralis* (Loew, 1844)	*intermedius* (Walker, 1834)
1	*Saussurea alpina* (L.) D.C.	FH	*Campiglossa sp. A*	*rhinthon* Walker, 1844
1	*Sonchus arvensis* L.	FH	*Tephritis dilacerata* (Loew, 1846)	*sonchi* Janzon, 1983 *egregius* Förster, 1841
1	*Tripolium pannonicum* Jacq.	FH	*Trupanea stellata* (Fuesslin, 1775)	*eudecipiens* (Özdikman, 2011) *intermedius* (Walker, 1834)

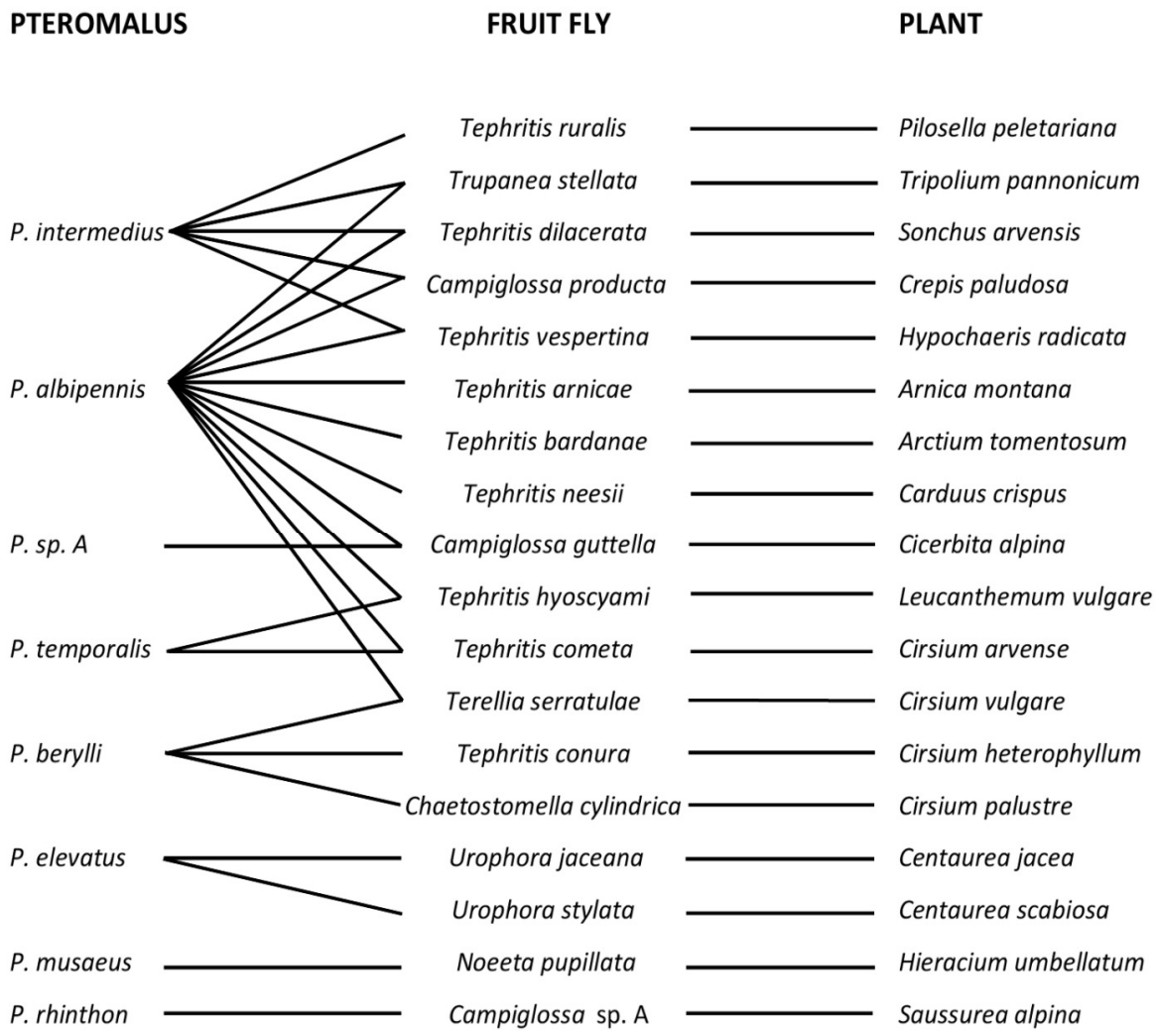

Figure 10 Relations among species of the three trophic levels, *Pteromalus* parasitoids, host fruit flies and host plants, based on the data in Table 5, of hatched insects from flower heads of Norwegian Asteraceae plants. Species first identified to *P. arnicae, P. eudecipiens, P. egregius* and *P. leucanthemi* are here treated under *P. albipennis*, and *P. sonchi* is treated under *P. intermedius*.

Discussion

The results indicate that 11 out of 18 *Pteromalus* species are reciprocally monophyletic, and well supported by the Bayesian analysis of COI. None of the sequence analyses were able to distinguish between *P. intermedius* and *P. sonchi*, or among *P. albipennis*, *P. arnicae*, *P. egregius*, *P. eudecipiens* and *P. leucanthemi*. This indicates that rather than being complexes of morphologically similar species, these constitute two species that are variable in morphology and/or in terms of host fruit fly preferences. Despite large intraspecific variations in the two gene trees, there was no indication of cryptic species, except for some support for the intraspecific divergence in *P. musaeus*.

Several nominal species within the same species boundaries

The Bayesian analyses of the COI and the ITS2 regions (Figure 7) showed that the species, *P. albipennis*, *P. arnicae*, *P. egregius*, *P. eudecipiens* and *P. leucanthemi,* were intermixed in one large monophyletic clade, with no species showing reciprocal monophyly. Neither the multispecies coalescent analysis (Figure 9) was able to distinguish among the species, but rather the opposite, it supported that they belong within the boundaries of a single species by giving no support to the intraspecific variation of the clade. In another case the two species, *P. intermedius* and *P. sonchi*, were intermixed in one single monophyletic clade in the COI gene tree, and separated by very small genetic distances (Table 3, Figure 7).

Because many of these species are nearly morphologically indistinguishable, the sequence analyses and morphology determinations are not necessary conflicting. In cases where morphology-based determinations have been nearly unachievable due to overlapping quantitative diagnostic characters, the species determinations have largely been based on host fruit fly relations. This involves *P. albipennis, P. arnicae* and *P. leucanthemi*, species that by Janzon (1984) were referred to as a complex of cryptic species. Polaszek *et al.* (2004) made a redescription of *P. leucanthemi* by comparing it to *P. albipennis*, and they pointed out the great similarities between the two species, and therefore stated that what they had

identified as *P. leucanthemi,* had been done with some reservation. Similarly, *P. sonchi* and *P. albipennis* are very close morphologically (Janzon 1986).

In contrast, the species *P. eudecipiens* and *P. egregius* are possible to identify by means of morphology. *P. egregius* differ from *P. albipennis* mainly in colouration and a somewhat smaller size, and according to Kurdjumov (1913) this might just be a polymorphic state of *P. albipennis*. On the other hand, *P. eudecipiens* differ from *P. albipennis* with a smaller size and a different gaster length/width ratio (Graham 1969). In a morphometric study, Baur (2002) conducted multivariate statistical methods on quantitative characters of *P. albipennis, P. eudecipiens* and *P. solidaginis* Graham & Gijswijt, 1991, revealing that *P. eudecipiens* differed from *P. albipennis* in size, but not shape. It is a known issue that characters may show variation due to environmental effects (Shingleton *et al.* 2007), e.g. size-related features will most likely be affected by the nutrition available during the larval stage. With a size change, body parts will usually change size in a slightly disproportionate manner, something called an allometric change (Gould 1966, Janzon 1986). The smaller size of *P. eudecipiens* may simply be an environmental effect, and the different gaster length/breadth ratio, due to an allometric change. This is possible because in this case *P. eudecipiens* developed from the relatively small host fruit fly, *Trupanea stellata*, hatched from the small flower heads of *Tripolium pannonicum* (Table 5). Not only variability in plant species or host insect species may be the cause of allometric changes, but also other conditions such as soil fertility, temperature, moisture, light, etc. (Shingleton *et al.* 2007). A size change due to environmental factors, which may cause allometric change, is something one should take into consideration when dealing with diagnostic characters.

It is important to notice that *P. eudecipiens* and *P. leucanthemi* are each only represented by one specimen in the sequence analysis, and that the possibility of these specimens being outliers will reduce the significance of the result regarding these species. Sequence data from more material is therefore needed before jumping to conclusions.

Cryptic species

The divergence thresholds on the two gene trees, set by the GMYC analysis (Figure 8), indicated a consistent divergence of two putative species within *P. musaeus*, and the multispecies coalescent analysis also gave some posterior probability support for this divergence (0.78). The singleton was collected in a malaise trap, with unknown host relations. The two specimens that constitute the other putative species, were not from the same hatching event, but hatched from different plants species at different locations in different years. One is hatched from *Hieracium aurentiacum* in relation with the host fruit fly, *Noeeta pupillata*, and the other were hatched from *Pilosella lactucella* (Table 5), probably also in relation with *Noeeta pupillata,* as this fruit fly is known to be associated with both *P. musaeus* and plants in the genus *Pilosella* (Janzon 1984).

The GMYC analysis (Figure 8) also indicated more than one putative species in *P. apum, P. berylli* and *P. intermedius*, but as these were not included in the ITS2 dataset, it is impossible to know whether this variation is caused by cryptic species, or not. Although ITS2 were sampled from both *P. intermedius* and *P. apum*, these specimens did unfortunately not yield any results.

Intraspecific variation

An issue with single locus species delimitation is that large intraspecific variation in gene phylogenies may not only be due to cryptic species, but occurs naturally as polymorphism within species (Funk & Omland 2003, Maddison 1997). Therefore, species trees and gene trees will often be incongruent (Maddison 1997), sometimes even for the most frequent gene tree topology ("The anomaly zone", Degnan & Rosenberg 2006). A solution to this problem can be to analyse multiple unlinked loci under The Multispecies Coalescent Model (Degnan and Rosenberg 2009, Heled & Drummond 2010), which will try to find a species tree topology that fits the evolutionary histories of all the analysed genes (Heled & Drummond 2010).

In this study, large intraspecific variations in COI are indicated by the gene tree (Figure 7a) and the within-species genetic distances (Table 4), especially in *P. elevatus* and the

P. albipennis clade. Large intraspecific variation in ITS2 is also indicated for the *P. albipennis* clade (Figure 7b). The GMYC analysis (Figure 8) suggested that five (COI) or six (ITS2) putative species occur in the *P. albipennis* clade, and three in *P. elevatus* (COI). However, these divergences were not supported by the Bayesian analysis conducted under the multispecies coalescent model (Figure 9), and by the fact that the two gene trees were largely incongruent for the internal topologies of the two clades. It is also noteworthy that no pattern in terms of host fruit fly preferences, or geographical occurrences, in *P. elevates* and the *P. albipennis* clade were detected that could explain the intraspecific variation (See Appendix 3). These results suggest that the intraspecific variation is not caused by the presence of cryptic species, but exists within the species as polymorphism. There may be at least four explanations to the polymorphism seen in these species.

First, polymorphism may be present in recently diverged species because loss of variability due to genetic drift (lineage sorting) has not had much time to sort out haplotypes (Maddison 1997). If what we see as one species really is a complex of young cryptic species, it is possible that these still share several haplotypes due to the short time of reproductive isolation. However, speciation in itself does not necessary account for the large intraspecific genetic distances. This may on the other hand be an indication on recently diverged species, or populations that have been isolated for a long time, having undergone speciation in reverse or secondary contact and therefore merged into a single lineage (Webb *et al.* 2011, Taylor 2006).

Second, it is well known that polymorphism in a species can be maintained over long time, if the effective population size has been large and stable, and the population have behaved panmictic. Linage sorting due to genetic drift will decrease in a large population with random mating, simply due to stochasticity (Maddison 1997, Kingsman 1982), and large intraspecific variation (deep coalescences) will be likely to occur (Kingsman 1982, Hogner *et al.* 2012). Both *P. albipennis* and *P. elevatus* attack fruit flies that are widely distributed, and it might therefore be that their effective population sizes are large. Historical demographic events, with variations in population size, can be estimated by conducting Tajimas D test (Tajima 1989, Hogner *et al.* 2012), which detects differences in allele frequency distribution from the expected, under the neutral model (Tajima 1989). A Bayesian analysis can also

estimate the population size through time, with the proper assignment of priors, such as mutation rate and root age (Drummond & Rambaut 2007).

Third, introgressive hybridization may cause patterns in gene trees that can be mistaken for incomplete lineage sorting where species cannot be delimited by reciprocal monophyly, but appear to be intermixed into several separate clades (Nicholls *et al.* 2012, Choleva *et al.* 2014). If these are morphologically indistinguishable sibling species, it may appear as large intraspecific variation. Hybridization occurs in 12.4% of European butterflies and is suggested to occur in around 10% of animal species (Mallet 2005), mainly in the youngest species, diverging between 2-5 Ma, with generally 2-6% divergence in mitochondrial DNA (Mallet 2005). Issues with introgression can be overcome by including more unlinked loci in the phylogenetic analysis, because unlinked loci during backcrossing will gradually be dispersed over the population, due to recombination (Nicholls *et al.* 2012). Therefore, haplotypes introduced to the gene pool by hybridization will have less influence on the phylogenetic inference (Nicholls *et al.* 2012). Furthermore, introgression can be tested by constructing a phylogenetic network (Nakhleh 2010, Yu *et al.* 2012, Wen *et al.* 2016), where data from several unlinked loci are analysed in a reticulate evolutionary context, i.e. where gene tree incongruences is considered to be caused by reticulations (Hybridization, horizontal gene transfer, etc.) in addition to incomplete lineage sorting.

Fourth, endosymbiotic microorganisms in the genus *Wolbachia*, living in close relations with members of many different arthropod groups, are known to affect the evolution of their hosts (Werren 1997). The most common effect on infected insects is cytoplasmic incompatibility (Hurst & Jiggins 2005). This is a reproductive incompatibility between sperm and egg, which can make infected males unable to successfully reproduce with uninfected females or females infected with another *Wolbachia* strain (Hurst & Jiggins 2005). This may cause reproductive isolation within the population, leading to polymorphism that can be maintained in the population for a long time, and may be difficult to distinguish from demographic effects, or that of speciation (Hurst & Jiggins 2005, Kvie *et al.* 2013). It is common to search for *Wolbachia* infection by amplification of *Wolbachia* DNA (Jeyaprakash & Hoy 2000; Kvie *et al.* 2013). Given infection, the impact on insect polymorphism can be tested by comparing gene histories of the insects and the endobionts present (Kvie *et al.* 2013). *Wolbachia* is already known from *P. puparum* and *P. vibulis* (Cook & Butcher 1999),

and to possibly affect the evolution of some members of Pteromalidae belonging to the genus *Nassonia* (Campbell *et al.* 1994).

Because these causes of polymorphism are all well documented, and believed to occur quite frequently, the possibility of a combination of these cannot be excluded, e.g. co-occurrence of introgression and incomplete lineage sorting (Wen *et al.* 2016). Sequence analyses of several unlinked loci could give answers to the intraspecific variation issue, through yielding knowledge on demographic history and reticulate evolution. This can be achieved by conducting a Bayesian analysis under the multispecies coalescent model, with the relevant and properly assigned priors, and by constructing a phylogenetic network (Nakhleh 2010, Yu *et al.* 2012, Wen *et al.* 2016). In this case the evolutionary impact of eventual endosymbionts should also be tested.

Host fruit fly relations

More than half of the fruit fly species are attacked by *P. albipennis*, and one fourth by *P. intermedius*, while *P. berylli, P. elevatus* and *P. temporalis* attack two or three species each (Figure 10). The species *P. musaeus, P. rhinthon* and *P. sp.A* are recorded from only a single host fruit fly each (Figure 10). As expected, the fruit fly species are recorded from only one host plant each (Figure 10, Table 5), except *Chaetorellia jaceae* which emerged from both *Centaurea jacea* and *Centaurea nigra*, and *Chaetostomella cylindrica* which emerged from both *Centaurea nigra* and *Cirsium palustre* (Table 5).

The study has shown that the three species, *P. arnicae, P. leucanthemi* and *P. sonchi*, which were thought to be monophagous, probably are part of two more widespread generalist species. It also indicates that the two species, *P. albipennis* and *P. intermedius,* have a broader range of host fruit fly preferences than previously expected. These patterns are contradictory to a general perception that parasitic Hymenoptera generalists mostly constitute complexes of cryptic species (Bickford *et al.* 2007, Hambäck *et al.* 2013).

Hatching experiences

This study focused on hatching insects from as many plants as possible, and the effort distributed on each plant was therefore mostly insufficient. For several of the plant species that yielded no specimens of *Pteromalus* or fruit flies (See Appendix 4), it might just be that they were sampled at the wrong date phenologically. Although no fruit flies emerged, larvae were detected in the flower heads of *Taraxacum* and *Tragopogon pratensis*. On the other hand, some plants were extensively investigated for larvae without finding any, and are most likely not inhabited by fruit flies in Norway, such as *Anthemis tinctoria*, *Carlina vulgaris*, *Cenecio vulgaris* and *Sonchus oleraceus*.

The parasitoid-host relations were inferred largely based on a one to one ratio of host and parasitoid species emerging together. In some hatching events, several fruit flies emerged together with the parasitoids, making it impossible to infer any relations (Table 5). In order to get more reliable and accurate results, it is at least two possible ways to improve the hatching method. First, a method used by Janzon (1980, 1983, 1984) involves dissecting the flowers and picking out the fruit fly larvae or pupae, identifying them and isolating the separate species before they hatch. This method is time consuming and it works only with endoparasitoids, but it yields accurate and qualitative results. Second, a quantitative approach can reveal the likely parasitoid-host relations, by increasing the sample size (number of hatching events), based on species of *Pteromalus* and fruit flies frequently emerging together. This method has the advantage over the first that it may cover a larger geographical scale, due to less effort put into each sample, and that it will yield a large insect material. For a serious study on parasitoid-host relations, a combination of the two may be the most convenient, where the first validates the relations recorded by the second.

Conclusion

Sequence analyses of COI and ITS2 indicates that one complex of five morphologically similar species, *P. albipennis*, *P. arnicae*, *P. egregius*, *P. eudecipiens* and *P. leucanthemi* are together located within the boundaries of one single species. The analysis of COI also indicates that two morphologically similar species, *P. intermedius* and *P. sonchi,* are located within the boundaries of another single species. Although most of these species are nearly morphologically indistinguishable, some are also achievable to identify by morphology, something that may be due to polymorphic varieties, and allometric change. In addition, an intraspecific divergence in the species, *P. musaeus*, were given some support by both COI and ITS2, indicating that it may consist of two closely related species. In addition, large intraspecific variation were seen in both the gene trees, that must be explained by something else than the presence of cryptic species, such as recent radiation or speciation in reverse, large population size, hybridization, or *Wolbachia* infection. Finally, this study has shown that the three Pteromalus species, *P. arnicae*, *P. leucanthemi* and *P. sonchi*, previously thought to be monophagous specialists, rather may be part of two widely distributed species with broad host fruit fly preferences.

In conclusion, the study shows that neither morphology, nor ecology, nor sequence data, are alone suitable for species delimitation in this group. However, the three approaches can do well together. It is therefore supporting the idea of an integrative taxonomy where a number of characters and traits from different approaches, such as morphology, ecology and DNA, are promoted to accurately delimit species (Dayrat 2005, Will *et al.* 2005, Padial *et al.* 2010, Schlick-Steiner *et al.* 2010). In this context it is noteworthy that species should be treated as testable hypotheses (Dominguez & Wheeler 1997), and their descriptions should be modified over time with the inclusion of new specimens and characters, and the adoption of new methods to the taxonomical work (Dominguez & Wheeler 1997).

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Table 1 Examined specimens of *Pteromalus* with collection data, and information about obtained sequence data of the mitochondrial COI and the nuclear ITS2 regions. Specimens are identified by a four digit accession number (Acc. no.). The obtained DNA sequences are given in base pair lengths. Unsuccessful sequencing, where no sequence data was obtained, is indicated by zero (0). Sequences included in the analyses are marked with asterisks (*). Information on collecting method (col. method) indicates which specimens are hached (Ex.) and from what substrate. Coordinates are given in decimal grades (Latitude/Longitude).

Acc. no.	Species	Sex	Collector	Date	Locality	Municipality	Latitude	Longitude	Method	COI	ITS2
4905	*Pteromalus albipennis*	M	Hansen, L. O.; Rindal, E.	01.10.2003	Bogerudmyra, Østensjøvannet	Oslo	59.881068	10.833603	Ex. Flower head of *Arctium tomentosum*	0	
4906	*Pteromalus albipennis*	M	Hansen, L. O.; Rindal, E.	01.10.2003	Bogerudmyra, Østensjøvannet	Oslo	59.881068	10.833603	Ex. Flower head of *Arctium tomentosum*	415 *	
4907	*Pteromalus temporalis*	F	Endrestøl, A.	08.07.2007	Kirkeby , Maridalen	Oslo	59.998434	10.759997	Ex. Flower head of *Arnica montana*	415 *	
4908	*Pteromalus albipennis*	F	Endrestøl, A.	08.07.2007	Kirkeby , Maridalen	Oslo	59.998434	10.759997	Ex. Flower head of *Arnica montana*	415 *	
4909	*Pteromalus albipennis*	F	Hansen, L. O.; Rindal, E.	01.08.2007	Kirkeby , Maridalen	Oslo	59.998434	10.759997	Ex. Flower head of *Cirsium heterophyllum*	415 *	
4910	*Pteromalus elevatus*	F	Hansen, L. O.	01.05.2005	Storøykilen , Oksenøya	Bærum	59.89419	10.60070	Ex. Flower head of *Centaurea scabiosa*	415 *	582 *
4911	*Pteromalus elevatus*	F	Hansen, L. O.	01.05.2005	Storøykilen , Oksenøya	Bærum	59.89419	10.60070	Ex. Flower head of *Centaurea scabiosa*	415 *	593 *
4912	*Pteromalus berylli*	F	Sørlibråten, O.	04.03.1997	Losbydalen	Lørenskog	59.8833	10.98890	Ex. Flower head of *Circium heterophyllum*	0	
4913	*Pteromalus berylli*	F	Endrestøl, A.; Olberg, S.	09.06.2006 - 27.07.2006	Lille Vestre Krutthus, Hovedøya	Oslo	59.89630	10.72571	Malaise trap	415 *	
4914	*Pteromalus musaeus*	F	Sørlibråten, O.	12.07.2002	Håkøybotn, Kvaløya	Tromsø	69.6300	18.7225	Sweep net	415 *	529 *
4915	*Pteromalus intermedius*	F	Wiig, G.	01.07.2004 - 31.07.2004	Furulia	Lillesand	58.24251	08.35921	Malaise trap	415 *	
4916	*Pteromalus intermedius*	F	Hansen, L. O.	01.07.2002 - 31.08.2002	Storøykilen , Oksenøya	Bærum	59.89419	10.60070	Malaise trap	0	
4917	*Pteromalus intermedius*	F	Hansen, L. O.	28.07.1989 - 30.07.1989	Rauer, Onsøy	Fredrikstad	59.221553	10.694183	Sweep net	0	
4918	*Pteromalus intermedius*	F	Sagvolden, B.	01.08.1992 - 31.08.1992	Vårviken	Rollag	60.0158	09.25940	Malaise trap	0	
4919	*Pteromalus intermedius*	F	Sagvolden, B.	01.08.1992 - 31.08.1992	Vårviken	Rollag	60.0158	09.25940	Malaise trap	415 *	0
4920	*Pteromalus berylli*	F	Hansen, L. O.	06.07.1991 - 02.08.1991	Kommersøya	Sand	59.515185	10.323728	Malaise trap	0	
4921	*Pteromalus chrysos*	F	Hansen, L. O.; Hanssen, O.	19.08.1995 - 01.10.1995	Verket	Hurum	59.6156	10.41610	Malaise trap	0	
4922	*Pteromalus chrysos*	M	Hansen, L. O.; Hanssen, O.	27.08.1995 - 15.09.1995	Melsomvik	Stokke	59.218084	10.346418	Window trap	0	
4923	*Pteromalus chrysos*	F	Fjeldså, A.	10.08.1984	Sandø	Tjøme	59.076656	10.466113	Sweep net	0	
4924	*Pteromalus patro*	M	Sund, K.	20.07.2003	Abborhøgda	Kongsvinger	60.1844	12.46020	Ex. Flower-head of *Arnica montana*	415	
4925	*Pteromalus patro*	M	Hansen, L. O.	20.07.2007	Sandø	Tjøme	59.08369	10.46310	Sweep net	0	
4926	*Pteromalus fasciatus*	F	Pedersen, J. R.	01.06.2006	Storøykilen , Oksenøya	Bærum	59.894198	10.599052	Ex. Potato-gall on *Quercus*	415	
4927	*Pteromalus apum*	F	Gammelmo, Ø.; Lønnve, O. J.	21.07.2005 - 26.08.2005	Hovedøya	Oslo	59.89529	10.74067	Malaise trap	415 *	0
4928	*Pteromalus apum*	F	Gammelmo, Ø.; Lønnve, O. J.	29.06.2005 - 21.07.2005	Hovedøya	Oslo	59.89529	10.74067	Malaise trap	415 *	0
4929	*Pteromalus apum*	F	Wiig, G.	01.09.2004 - 30.09.2004	Furulia	Lillesand	58.24251	08.35921	Malaise trap	613 *	0
4930	*Pteromalus apum*	F	Endrestøl, A.; Olberg, S.	19.07.2005 - 24.08.2005	Lindøya	Oslo	59.89147	10.71670	Malaise trap	635 *	
4931	*Pteromalus apum*	F	Wiig, G.	01.07.2004 - 31.07.2004	Furulia	Lillesand	58.24251	08.35921	Malaise trap	620 *	0
4932	*Pteromalus sp.*	M	Hansen, L. O.	01.05.1994	Underlia	Drammen	59.75551	10.17677	Ex. Gall (Dipl. rosae) on *Rosa*	401	
4933	*Pteromalus bedeguaris*	F	Hansen, L. O.	01.05.1994	Underlia	Drammen	59.75551	10.17677	Ex. Gall (Dipl. rosae) on *Rosa*	0	
4934	*Pteromalus berylli*	F	Hansen, L. O.	16.07.2003	Ørekroken, Kjerkøy	Hvaler	59.032842	11.010232	Sweep net	415 *	
4935	*Pteromalus cioni*	F	Hansen, L. O.	01.07.2002 - 31.08.2002	Storøykilen , Oksenøya	Bærum	59.894654	10.600646	Malaise trap	401 *	
4936	*Pteromalus cioni*	F	Endrestøl, A.; Olberg, S.	19.07.2005 - 24.08.2005	Lindøya	Oslo	59.89147	10.71670	Malaise trap	376 *	
4937	*Pteromalus cioni*	F	Hansen, L. O.	01.09.1991 - 26.10.1991	Tofteholmen	Hurum	59.5158	10.55940	Malaise trap	401 *	
4938	*Pteromalus dispar*	F	Sørlibråten, O.	26.06.2005	Kongsvinger Festningen	Kongsvinger	60.199451	12.011664	Ex. *Viscaria vulgaris*	415 *	
4939	*Pteromalus dispar*	F	Sørlibråten, O.	01.04.2000	Holtsætra, Holmarka	Ullensaker	60.0786	11.30170	Ex. Flower head of *Hieraceum umbellatum*	0	
4940	*Pteromalus dispar*	F	Endrestøl, A.; Olberg, S.	19.07.2005 - 24.08.2005	Lindøya	Oslo	59.89147	10.71670	Malaise trap	401	
4941	*Pteromalus dispar*	F	Olsen, K. M.; Reise, S.	19.07.2002 - 26.08.2002	Dausjøen , Maridalen	Oslo	60.01257	10.78828	Malaise trap	0	
4942	*Pteromalus dispar*	F	Endrestøl, A.; Olberg, S.	25.07.2006 - 04.09.2006	Rodeløkken, Bygdøy	Oslo	59.91499	10.69019	Malaise trap	0	
4943	*Pteromalus hieracii*	F	Hansen, L. O.	11.08.1990	Langøya, Våle	Re	59.499817	10.366113	Sweep net	0	
4944	*Pteromalus platyphilus*	F	Endrestøl, A.; Olberg, S.	01.06.2005 - 19.07.2005	Lindøya	Oslo	59.89147	10.71670	Malaise trap	415	
4945	*Pteromalus platyphilus*	F	Falck, Morten; Hansen, L. O.	01.08.1995 - 31.08.1995	Manglerud, Østensjøvannet	Oslo	59.89231	10.82648	Malaise trap	0	
4946	*Pteromalus puparum*	M	Sørlibråten, O.	25.09.1996	Egner	Sørum	60.0694	11.24530	Ex. Pupa of *Aglais urctica*	0	
4947	*Pteromalus puparum*	M	Sørlibråten, O.	01.05.1997	Egner	Sørum	60.0694	11.24530	Ex. Pupa of *Vanessa antiopa*	0	
4948	*Pteromalus puparum*	M	Sørlibråten, O.	01.03.1997	Egner	Sørum	60.0694	11.24530	Ex. Pupa of *Vanessa antiopa*	415	
4949	*Pteromalus elevatus*	F	Endrestøl, A.; Olberg, S.	01.06.2005 - 19.07.2005	Lindøya	Oslo	59.89147	10.71670	Malaise trap	415 *	571 *
4950	*Pteromalus sp.*	M	Hansen, L. O.; Rindal, E.	29.07.2004 - 14.09.2004	Starmoen	Elverum	60.84990	11.69168	Malaise trap	626	
4951	*Pteromalus albipennis*	F	Hansen, L. O.	01.08.2002	Storøykilen, Oksenøya	Bærum	59.894654	10.600646	Sweep net	401 *	
4952	*Pteromalus sp.*	M	Christensen, R.	15.01.2004	Gamle moss fyllplass	Moss	59.458408	10.688445	Ex. Larva of *Coleophora tripoli*	0	
4953	*Pteromalus sp.*	M	Christensen, R.	15.01.2004	Gamle moss fyllplass	Moss	59.458408	10.688445	Ex. Larva of *Coleophora tripoli*	415	

4954	*Pteromalus sequester*	F	Fjellberg, A.; Hanssen, O.	04.07.1995 - 26.07.1995	Midtre Bolerne	Nøtterøy	59.211759	10.542142	Malaise trap	0	
4955	*Pteromalus sequester*	F	Hansen, L. O.	16.07.2003 - 15.08.2003	Ørekroken, Kjerkøy	Hvaler	59.032913	11.009910	Malaise trap	0	
4956	*Pteromalus temporalis*	F	Bakke, A.	12.07.1953	Otta	Sel	61.772499	09.539553	Sweep net	0	
4957	*Pteromalus tibiellus*	F	Berg, Y.; Hansen, L. O.	01.07.1994 - 31.07.1994	Ryggsætra, Hagatjern, Mjøndalen	Nedre Eiker	59.73344	10.04722	Malaise trap	0	
4958	*Pteromalus egregius*	F	Wiig, G.	01.08.2004 - 30.09.2004	Furulia	Lillesand	58.24251	08.35921	Malaise trap	415 *	
4978	*Pteromalus chlorogaste*	F	Endrestøl, A.	09.05.2008 - 17.06.2008	Ekebergskråningen	Oslo	59.89414	10.75956	Malaise trap	0	
4979	*Pteromalus chlorogaste*	F	Endrestøl, A.	09.05.2008 - 17.06.2008	Ekebergskråningen	Oslo	59.89414	10.75956	Malaise trap	0	
4980	*Pteromalus chlorogaste*	F	Endrestøl, A.	09.05.2008 - 17.06.2008	Ekebergskråningen	Oslo	59.89414	10.75956	Malaise trap	0	
4981	*Pteromalus hieracii*	F	Endrestøl, A.	09.05.2008 - 17.06.2008	Ekebergskråningen	Oslo	59.89414	10.75956	Malaise trap	415	
4982	*Pteromalus chlorogaste*	F	Endrestøl, A.	09.05.2008 - 17.06.2008	Ekebergskråningen	Oslo	59.89414	10.75956	Malaise trap	0	
4983	*Pteromalus sequester*	F	Berg, Y.; Hansen, L. O.	01.08.1994 - 31.08.1994	Ryggsætra, Hagatjern, Mjøndalen	Nedre Eiker	59.73347	10.04595	Malaise trap	0	
4984	*Pteromalus sequester*	F	Hansen, L. O.	12.07.2008 - 03.08.2008	Ryggkollen, Mjøndalen	Nedre Eiker	59.75390	10.04912	Malaise trap	0	
5000	*Pteromalus albipennis*	F	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.660187	10.768557	Ex. Flower head of *Cirsium arvense*	458 *	477 *
5001	*Pteromalus albipennis*	F	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.660187	10.768557	Ex. Flower head of *Cirsium arvense*	0	
5002	*Pteromalus sp. A*	F	Lindemann, J. P.	14.04.2015	Gjærdal	Røyken	59.68945	10.41166	Ex. Flower head of *Cirsium arvense*	509 *	
5003	*Pteromalus albipennis*	F	Lindemann, J. P.	14.04.2015	Gjærdal	Røyken	59.68945	10.41166	Ex. Flower head of *Cirsium arvense*	481 *	
5004	*Pteromalus sp. A*	F	Lindemann, J. P.	14.04.2015	Gjærdal	Røyken	59.68945	10.41166	Ex. Flower head of *Cirsium arvense*	624 *	
5005	*Pteromalus elevatus*	F	Lindemann, J. P.	14.04.2015	Gjærdal	Røyken	59.68945	10.41166	Ex. Flower head of *Centaurea jacea*	478 *	569 *
5006	*Pteromalus elevatus*	M	Lindemann, J. P.	14.04.2015	Gjærdal	Røyken	59.68945	10.41166	Ex. Flower head of *Centaurea jacea*	441 *	
5007	*Pteromalus elevatus*	F	Lindemann, J. P.	03.09.2014	Østensjøvannet	Ås	59.689068	10.814560	Ex. Flower head of *Centaurea jacea*	474 *	569 *
5008	*Pteromalus elevatus*	F	Lindemann, J. P.	03.09.2014	Østensjøvannet	Ås	59.689068	10.814560	Ex. Flower head of *Centaurea jacea*	476 *	
5009	*Pteromalus elevatus*	M	Lindemann, J. P.	03.09.2014	Østensjøvannet	Ås	59.689068	10.814560	Ex. Flower head of *Centaurea jacea*	0	
5010	*Pteromalus chlorospilus*	F	Lindemann, J. P.	15.05.2015	Østensjøvannet	Ås	59.689068	10.814560	Ex. Flower head of *Centaurea jacea*	0	
5011	*Pteromalus chlorospilus*	M	Lindemann, J. P.	14.05.2015	Årungen	Ås	59.691196	10.734387	Ex. Flower head of *Centaurea jacea*	476 *	
5012	*Pteromalus temporalis*	M	Lindemann, J. P.	08.08.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Cirsium arvense*	481 *	
5013	*Pteromalus berylli*	F	Lindemann, J. P.	01.07.2015	Granberg	Eidsberg	59.5469	11.23920	Ex. Flower head of *Cirsium hetrophylum*	0	
5014	*Pteromalus berylli*	F	Lindemann, J. P.	01.07.2015	Granberg	Eidsberg	59.5469	11.23920	Ex. Flower head of *Cirsium hetrophylum*	480 *	
5015	*Pteromalus caudiger*	F	Lindemann, J. P.	01.07.2015	Granberg	Eidsberg	59.5469	11.23920	Ex. Flower head of *Cirsium hetrophylum*	0	
5016	*Pteromalus albipennis*	M	Lindemann, J. P.	01.07.2015	Granberg	Eidsberg	59.5469	11.23920	Ex. Flower head of *Cirsium hetrophylum*	0	
5017	*Pteromalus intermedius*	F	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	08.61103279	Ex. Flower head of *Hypochaeris radicata*	476 *	
5018	*Pteromalus intermedius*	F	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	08.61103279	Ex. Flower head of *Hypochaeris radicata*	474 *	
5019	*Pteromalus albipennis*	F	Lindemann, J. P.	24.06.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Hypochaeris radicata*	0	
5020	*Pteromalus sp.*	M	Lindemann, J. P.	24.06.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Hypochaeris radicata*	0	
5021	*Pteromalus sp.*	M	Lindemann, J. P.	24.06.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Hypochaeris radicata*	0	
5022	*Pteromalus berylli*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium vulgare*	402 *	
5023	*Pteromalus berylli*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium vulgare*	455 *	
5024	*Pteromalus albipennis*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium vulgare*	0	
5025	*Pteromalus berylli*	M	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium vulgare*	0	
5026	*Pteromalus berylli*	M	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium vulgare*	445 *	
5027	*Pteromalus intermedius*	M	Lindemann, J. P.	26.06.2015	Maridalen, Kirkeruinen	Oslo	59.996407	10.759721	Ex. Flower head of *Pilosella peletariana*	474 *	
5028	*Pteromalus intermedius*	M	Lindemann, J. P.	13.06.2014	Søm, Ruakerkilen	Grimstad	58.382908	08.71535713	Ex. Flower head of *Pilosella peletariana*	474 *	
5029	*Pteromalus sp.*	M	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	08.61103279	Ex. Flower head of *Hieracium sp.*	480 *	
5030	*Pteromalus sp.*	M	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	08.61103279	Ex. Flower head of *Hieracium sp.*	0	
5031	*Pteromalus egregius*	F	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	08.61103279	Ex. Flower head of *Hieracium sp.*	603 *	563 *
5032	*Pteromalus egregius*	F	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	08.61103279	Ex. Flower head of *Hieracium sp.*	0	
5033	*Pteromalus berylli*	M	Lindemann, J. P.	17.07.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Cirsium palustre*	482 *	
5034	*Pteromalus berylli*	F	Lindemann, J. P.	17.07.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Cirsium palustre*	455 *	
5035	*Pteromalus dispar*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium palustre*	0	
5036	*Pteromalus dispar*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium palustre*	618 *	
5037	*Pteromalus dispar*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium palustre*	0	
5038	*Pteromalus dispar*	M	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium palustre*	0	

5039	*Pteromalus dispar*	M	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium palustre*	652 *	
5040	*Pteromalus berylli*	M	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium palustre*	475 *	
5041	*Pteromalus albipennis*	F	Lindemann, J. P.	16.04.2015	Årungen	Ås	59.67279	10.75233	Ex. Flower head of *Arctium sp.*	476 *	
5042	*Pteromalus sp.*	F	Lindemann, J. P.	16.04.2015	Årungen	Ås	59.67279	10.75233	Ex. Flower head of *Arctium sp.*	0	
5043	*Pteromalus sp.*	M	Lindemann, J. P.	16.04.2015	Årungen	Ås	59.67279	10.75233	Ex. Flower head of *Arctium sp.*	0	
5044	*Pteromalus albipennis*	F	Lindemann, J. P.	16.04.2015	Årungen	Ås	59.67279	10.75233	Ex. Flower head of *Arctium sp.*	0	
5045	*Pteromalus albipennis*	M	Lindemann, J. P.	15.04.2015	Maridalen, Kirkeruinen	Oslo	59.997283	10.767667	Ex. Flower head of *Arctium tomentosum*	0	
5046	*Pteromalus albipennis*	M	Lindemann, J. P.	15.04.2015	Maridalen, Kirkeruinen	Oslo	59.997283	10.767667	Ex. Flower head of *Arctium tomentosum*	474 *	
5047	*Pteromalus albipennis*	F	Lindemann, J. P.	15.04.2015	Maridalen, Kirkeruinen	Oslo	59.997283	10.767667	Ex. Flower head of *Arctium tomentosum*	0	
5048	*Pteromalus sp.*	M	Lindemann, J. P.	31.03.2014	Årungen	Ås	59.683724	10.75316874	Ex. Flower head *Hieracium umbellatum*	0	
5049	*Pteromalus musaeus*	F	Lindemann, J. P.	31.03.2014	Årungen	Ås	59.683724	10.75316874	Ex. Flower head *Hieracium umbellatum*	476 *	541 *
5050	*Pteromalus semotus*	F	Lindemann, J. P.	17.04.2015	Fevik, Strand	Grimstad	58.371821	08.669557	Ex. Flower head of *Centaurea nigra*	652 *	
5051	*Pteromalus semotus*	F	Lindemann, J. P.	17.04.2015	Fevik, Strand	Grimstad	58.371821	08.669557	Ex. Flower head of *Centaurea nigra*	586 *	
5052	*Pteromalus semotus*	F	Lindemann, J. P.	17.04.2015	Fevik, Strand	Grimstad	58.371821	08.669557	Ex. Flower head of *Centaurea nigra*	648 *	
5053	*Pteromalus semotus*	M	Lindemann, J. P.	17.04.2015	Fevik, Strand	Grimstad	58.371821	08.669557	Ex. Flower head of *Centaurea nigra*	652 *	
5054	*Pteromalus semotus*	M	Lindemann, J. P.	17.04.2015	Fevik, Strand	Grimstad	58.371821	08.669557	Ex. Flower head of *Centaurea nigra*	0	
5055	*Pteromalus sonchi*	F	Lindemann, J. P.	02.09.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Sonchus arvensis*	626 *	
5056	*Pteromalus sonchi*	F	Lindemann, J. P.	02.09.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Sonchus arvensis*	447 *	558 *
5057	*Pteromalus egregius*	F	Lindemann, J. P.	02.09.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Sonchus arvensis*	478 *	479 *
5058	*Pteromalus sonchi*	F	Lindemann, J. P.	02.09.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Sonchus arvensis*	474 *	
5059	*Pteromalus sonchi*	F	Lindemann, J. P.	02.09.2014	Herumveien	Ås	59.635815	10.776139	Ex. Flower head of *Sonchus arvensis*	0	
5065	*Pteromalus intermedius*	M	Lindemann, J. P.	07.07.2015	Maridalen, Kirkeruinen	Oslo	59.995830	10.756438	Ex. Flower head of *Arnica montana*	0	
5066	*Pteromalus rhinthon*	F	Lindemann, J. P.	17.06.2015	Aurlandsdalen, Sinjarheim	Oslo	60.857243	07.408538	Ex. Flower head of *Saussurea alpina*	477 *	
5067	*Pteromalus albipennis*	F	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	0	
5068	*Pteromalus sp.*	M	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	0	
5069	*Pteromalus sp.*	M	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	419 *	
5070	*Pteromalus albipennis*	F	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	608 *	549 *
5071	*Pteromalus egregius*	M	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	476 *	465 *
5072	*Pteromalus albipennis*	F	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	476 *	541 *
5073	*Pteromalus albipennis*	M	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	409 *	
5074	*Pteromalus albipennis*	M	Lindemann, J. P.	22.07.2015	Kilehagen	Ås	59.659979	10.768309	Ex. Flower head of *Carduus crispus*	458 *	0
5075	*Pteromalus eudecipiens*	M	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	0	
5076	*Pteromalus eudecipiens*	M	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	477 *	562 *
5077	*Pteromalus eudecipiens*	F	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	0	
5078	*Pteromalus intermedius*	F	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	473 *	
5079	*Pteromalus sp.*	M	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	0	
5080	*Pteromalus intermedius*	F	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	0	
5081	*Pteromalus eudecipiens*	F	Lindemann, J. P.	12.07.2015	Søm, Hasseltangen	Grimstad	58.394260	08.726767	Ex. Flower head of *Tripolium pannonicum*	0	
5082	*Pteromalus sp.*	M	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	0	
5083	*Pteromalus sp.*	M	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	0	
5084	*Pteromalus leucanthem.*	F	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	474 *	503 *
5085	*Pteromalus leucanthem.*	F	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	0	
5086	*Pteromalus leucanthem.*	F	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	0	
5087	*Pteromalus leucanthem.*	F	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	0	
5088	*Pteromalus leucanthem.*	F	Lindemann, J. P.	01.07.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Leucharitemum vulgare*	0	
5089	*Pteromalus leucanthem.*	F	Lindemann, J. P.	24.06.2015	Levermyr, Furuliveien	Grimstad	58.346529	08.593525	Ex. Flower head of *Hypochaeris radicata*	0	
5090	*Pteromalus patro*	F	Lindemann, J. P.	02.09.2014	Årungen	Ås	59.672028	10.75056906	Ex. Flower head of *Arctium tomentosum*	0	
5091	*Pteromalus albipennis*	M	Lindemann, J. P.	02.09.2014	Årungen	Ås	59.672028	10.75056906	Ex. Flower head of *Arctium tomentosum*	476 *	
5092	*Pteromalus egregius*	F	Lindemann, J. P.	27.08.2014	Bjerkeli	Rakkestad	59.379514	11.270983	Ex. Flower head of *Cirsium arvense*	475 *	534 *
5093	*Pteromalus albipennis*	F	Lindemann, J. P.	01.04.2014	Dybedal	Grimstad	58.351121	08.584965	Ex. Flower head of *Cirsium arvense*	0	
5094	*Pteromalus intermedius*	F	Lindemann, J. P.	01.04.2014	Dybedal	Grimstad	58.351121	08.584965	Ex. Flower head of *Cirsium arvense*	475 *	

6389	*Pteromalus albipennis*	F	Hansen, L. O.	08.31.2015	Movann	Oslo	60.036918	10.808821	Ex. Flower head of *Cicerbita alpina*	596 *	
6390	*Pteromalus sp. A*	F	Hansen, L. O.	31.08.2015	Movann	Oslo	60.036918	10.808821	Ex. Flower head of *Cicerbita alpina*	657 *	
6391	*Pteromalus sp. A*	F	Hansen, L. O.	31.08.2015	Movann	Oslo	60.036918	10.808821	Ex. Flower head of *Cicerbita alpina*	628 *	
6392	*Pteromalus sp. A*	M	Hansen, L. O.	31.08.2015	Movann	Oslo	60.036918	10.808821	Ex. Flower head of *Cicerbita alpina*	658 *	
6393	*Pteromalus sp. A*	M	Hansen, L. O.	31.08.2015	Movann	Oslo	60.036918	10.808821	Ex. Flower head of *Cicerbita alpina*	0	
6394	*Pteromalus intermedius*	F	Hansen, L. O.	14.07.2013	Slåttemyra	Nittedal	60.042618	10.831311	Ex. Flower head of *Crepis paludosa*	252	0
6395	*Pteromalus sp.*	M	Hansen, L. O.	14.07.2013	Slåttemyra	Nittedal	60.042618	10.831311	Ex. Flower head of *Crepis paludosa*	190	
6396	*Pteromalus sp.*	M	Hansen, L. O.	14.07.2013	Slåttemyra	Nittedal	60.042618	10.831311	Ex. Flower head of *Crepis paludosa*	611 *	
6397	*Pteromalus albipennis*	M	Hansen, L. O.	14.07.2013	Slåttemyra	Nittedal	60.042618	10.831311	Ex. Flower head of *Crepis paludosa*	613 *	
6398	*Pteromalus albipennis*	F	Hansen, L. O.	14.07.2013	Slåttemyra	Nittedal	60.042618	10.831311	Ex. Flower head of *Crepis paludosa*	488 *	
6399	*Pteromalus sp.*	M	Hansen, L. O.; Thuroczy, C.	26.04.2015	Krokstad, Brekke	Nedre Eiker	59.764088	09.983982	Ex. Stem of *Centaurea scabiosa*	477	
6402	*Pteromalus hieracii*	M	Hansen, L. O.; Thuroczy, C.	26.04.2015	Krokstad, Brekke	Nedre Eiker	59.764088	09.983982	Ex. Stem of *Centaurea scabiosa*	125	
6403	*Pteromalus sp.*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Stem of *Centaurea jacea*	598	
6409	*Pteromalus elevatus*	F	Hansen, L. O.; Thuroczy, C.	26.04.2015	Krokstad, Brekke	Nedre Eiker	59.764088	09.983982	Ex. Flower head of *Centaurea scabiosa*	477 *	582 *
6410	*Pteromalus elevatus*	F	Hansen, L. O.; Thuroczy, C.	26.04.2015	Krokstad, Brekke	Nedre Eiker	59.764088	09.983982	Ex. Flower head of *Centaurea scabiosa*	605 *	
6411	*Pteromalus elevatus*	F	Hansen, L. O.; Thuroczy, C.	26.04.2015	Krokstad, Brekke	Nedre Eiker	59.764088	09.983982	Ex. Flower head of *Centaurea scabiosa*	480 *	
6414	*Pteromalus arnicae*	M	Hansen, L. O.; Magnussen, T.	12.06.2014	Maridalen, Kirkeby	Oslo	59.996123	10.756871	Ex. Flower head of *Arnica montana*	0	
6415	*Pteromalus temporalis*	M	Hansen, L. O.; Magnussen, T.	12.06.2014	Maridalen, Kirkeby	Oslo	59.996123	10.756871	Ex. Flower head of *Arnica montana*	0	
6416	*Pteromalus arnicae*	F	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	611 *	471 *
6417	*Pteromalus sp.*	M	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	612 *	
6418	*Pteromalus arnicae*	F	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	134	
6419	*Pteromalus sonchi*	F	Hansen, L. O.	08.10.2014	Bokerøya	Svelvik	59.590873	10.406734	Ex. Flower head of *Sonchus arvensis*	605 *	
6420	*Pteromalus sonchi*	F	Hansen, L. O.	08.10.2014	Bokerøya	Svelvik	59.590873	10.406734	Ex. Flower head of *Sonchus arvensis*	580 *	
6421	*Pteromalus sonchi*	M	Hansen, L. O.	08.10.2014	Bokerøya	Svelvik	59.590873	10.406734	Ex. Flower head of *Sonchus arvensis*	600 *	
6422	*Pteromalus sp.*	M	Hansen, L. O.	08.10.2014	Bokerøya	Svelvik	59.590873	10.406734	Ex. Flower head of *Sonchus arvensis*	0	
6423	*Pteromalus sp.*	M	Hansen, L. O.	08.10.2014	Bokerøya	Svelvik	59.590873	10.406734	Ex. Flower head of *Sonchus arvensis*	0	
6424	*Pteromalus fasciatus*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	0	
6425	*Pteromalus elevatus*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	599 *	
6426	*Pteromalus cf. vibulens*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	128	
6427	*Pteromalus elevatus*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	603 *	580 *
6428	*Pteromalus elevatus*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	598 *	567 *
6429	*Pteromalus musaeus*	F	Hansen, L. O.	20.06.2011	Åsbygda, Furuset	Stange	60.73157	11.36355	Ex. Flower head of *Hieracium lactucella*	593 *	540 *
6430	*Pteromalus sp.*	M	Hansen, L. O.	20.06.2011	Åsbygda, Furuset	Stange	60.73157	11.36355	Ex. Flower head of *Hieracium lactucella*	131	
6431	*Pteromalus sp.*	M	Hansen, L. O.	20.06.2011	Åsbygda, Furuset	Stange	60.73157	11.36355	Ex. Flower head of *Hieracium lactucella*	131	
6432	*Pteromalus sp.*	M	Hansen, L. O.	20.06.2011	Åsbygda, Furuset	Stange	60.73157	11.36355	Ex. Flower head of *Hieracium lactucella*	0	
6434	*Pteromalus temporalis*	F	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	0	
6435	*Pteromalus sp.*	M	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	0	
6436	*Pteromalus elevatus*	F	Hansen, L. O.; Thuroczy, C.	26.04.2015	Krokstad, Brekke	Nedre Eiker	59.764088	09.983982	Ex. Flower head of *Centaurea scabiosa*	565 *	
6437	*Pteromalus elevatus*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	604 *	
6438	*Pteromalus sp.*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Hellesmo	Tjøme	59.073333	10.398691	Ex. Flower head of *Centaurea scabiosa*	120	
6439	*Pteromalus caudiger*	M	Hansen, L. O.	25.04.2012	Prinsdal	Oslo	59.830264	10.816471	Ex. Flower head of *Cirsium hetrophyllum*	0	
6440	*Pteromalus caudiger*	M	Hansen, L. O.	25.04.2012	Prinsdal	Oslo	59.830264	10.816471	Ex. Flower head of *Cirsium hetrophyllum*	0	
6441	*Pteromalus caudiger*	F	Hansen, L. O.	25.04.2012	Prinsdal	Oslo	59.830264	10.816471	Ex. Flower head of *Cirsium hetrophyllum*	0	
6442	*Pteromalus caudiger*	F	Hansen, L. O.	25.04.2012	Prinsdal	Oslo	59.830264	10.816471	Ex. Flower head of *Cirsium hetrophyllum*	118	
6443	*Pteromalus caudiger*	F	Hansen, L. O.	25.04.2012	Prinsdal	Oslo	59.830264	10.816471	Ex. Flower head of *Cirsium hetrophyllum*	116	
6446	*Pteromalus hieracii*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	588	
6447	*Pteromalus albipennis*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	611 *	561 *
6448	*Pteromalus elevatus*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	482 *	567 *
6449	*Pteromalus elevatus*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	593 *	
6450	*Pteromalus sp.*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	598	
6451	*Pteromalus elevatus*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	569 *	

6452	*Pteromalus elevatus*	M	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	609 *	
6453	*Pteromalus elevatus*	F	Hansen, L. O.; Magnussen, T.	07.10.2014	Moutmarka	Tjøme	59.064751	10.399646	Ex. Flower head of *Centaurea jacea*	603 *	
6454	*Pteromalus arnicae*	F	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	120	
6455	*Pteromalus sp.*	M	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	0	
6456	*Pteromalus arnicae*	F	Hansen, L. O.; Bjureke, K	07.07.2011	Lier, Portnerboligen Lier	Kongsvinger	60.15055	12.03541	Ex. Flower head of *Arnica montana*	599 *	521 *
6457	*Pteromalus sp.*	M	Hansen, L. O.	01.05.2006	Bygdøy, Bygdøy Kongsgård	Oslo	59.9122	10.67940	Ex. Flower head of *Arctium tomentosum*	0	
6458	*Pteromalus sp.*	M	Hansen, L. O.	01.05.2006	Bygdøy, Bygdøy Kongsgård	Oslo	59.9122	10.67940	Ex. Flower head of *Arctium tomentosum*	0	
6464	*Pteromalus semotus*	F	Lindemann, J. P.	17.04.2015	Fevik, Fevikkilen	Grimstad	58.371821	8.669557	Ex. Flower head of *Centaurea nigra*	658 *	
6465	*Pteromalus semotus*	F	Lindemann, J. P.	17.04.2015	Fevik, Fevikkilen	Grimstad	58.371821	8.669557	Ex. Flower head of *Centaurea nigra*	658 *	
6466	*Pteromalus semotus*	F	Lindemann, J. P.	17.04.2015	Fevik, Fevikkilen	Grimstad	58.371821	8.669557	Ex. Flower head of *Centaurea nigra*	398	
6467	*Pteromalus intermedius*	F	Lindemann, J. P.	28.06.2014	Rønnes, Tollholtheia	Grimstad	58.340888	8.61103279	Ex. Flower head of *Hypochaeris radicata*	608 *	
6468	*Pteromalus beryili*	F	Lindemann, J. P.	16.04.2015	Kilehagen	Ås	59.66055	10.76805	Ex. Flower head of *Cirsium vulgare*	486 *	
6476	*Pteromalus albipennis*	F	Hansen, L. O.	01.05.2006	Bygdøy, Bygdøy Kongsgård	Oslo	59.9122	10.67940	Ex. Flower head of *Arctium tomentosum*	0	
6477	*Pteromalus albipennis*	F	Hansen, L. O.	01.05.2006	Bygdøy, Bygdøy Kongsgård	Oslo	59.9122	10.67940	Ex. Flower head of *Arctium tomentosum*	0	
6478	*Pteromalus albipennis*	F	Hansen, L. O.	01.05.2006	Bygdøy, Bygdøy Kongsgård	Oslo	59.9122	10.67940	Ex. Flower head of *Arctium tomentosum*	0	

Appendix 2

Table 1 PCR protocol for the chemicals used in the amplification of the nuclear ITS2 region. The Polymerase, Platnium Taq, was used, and the forward primer (Primer F) FFA and reverse primer (Primer R) ITS4_R were used.

Reagent	Reagent concentration	Unit	Volum (µl)	Master mix (µl)	Final consentration		Optimal concentration
dH2O			7.9	286.77	-		
Buffer	10	X	1.5	54.45	1	X	1X
$MgCl_2$	50	mM	0.5	18.15	1.66667	mM	1.5 mM
dNTP	20	mM	0.5	18.15	0.66667	mM	0.6 mM
Primer F	10	µM	0.75	27.225	0.5	µM	0.5 µM
Primer R	10	µM	0.75	27.225	0.5	µM	0.5 µM
DMSO	100	%		0	0	%	3%
Polymerase	5	U/µl	0.1	3.63	0.03333	U/µl	defined by sup
DNA		ng/µl	3	-	0	ng/µl	~50 ng
Total (µl)			15	435.6			

Table 2 PCR program used in the amplification of the nuclear ITS2 region.

PCR-program		
Temp	Duration	Cycles
94	2 min	1
96	30 s	
65	30 s	10
72	45 s	
96	30 s	
55	30 s	25
72	45 s	
72	7 min	1

Appendix 3

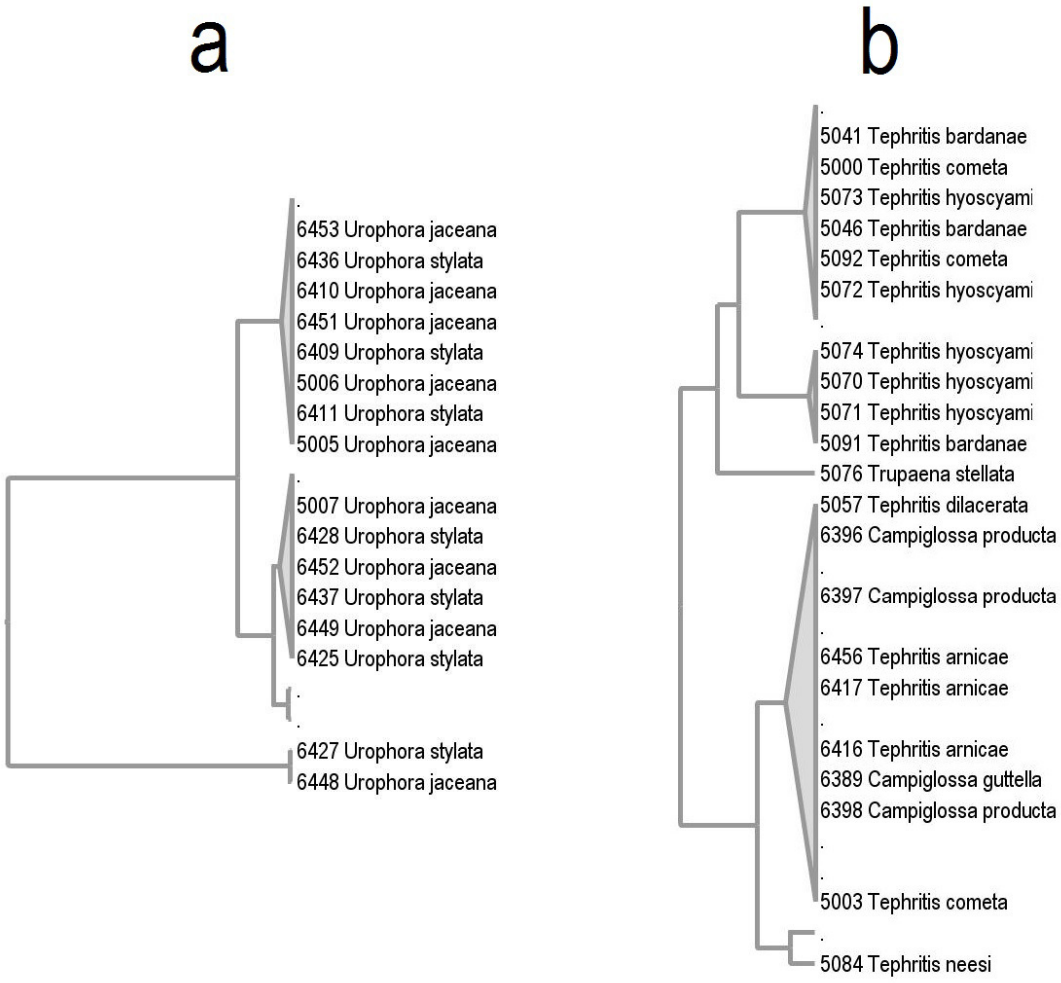

Figure 1 Clades of *Pteromalus elevatus* **(a)** and *Pteromalus albipennis* **(b)** from a Bayesian gene tree of the mitochondrial COI, where tips are labelled according to the host fruit fly species of the corresponding *Pteromalus* specimen. Note that there are no clear patterns of host fruit fly preferences that can explain the intraspecific variation within the two clades.

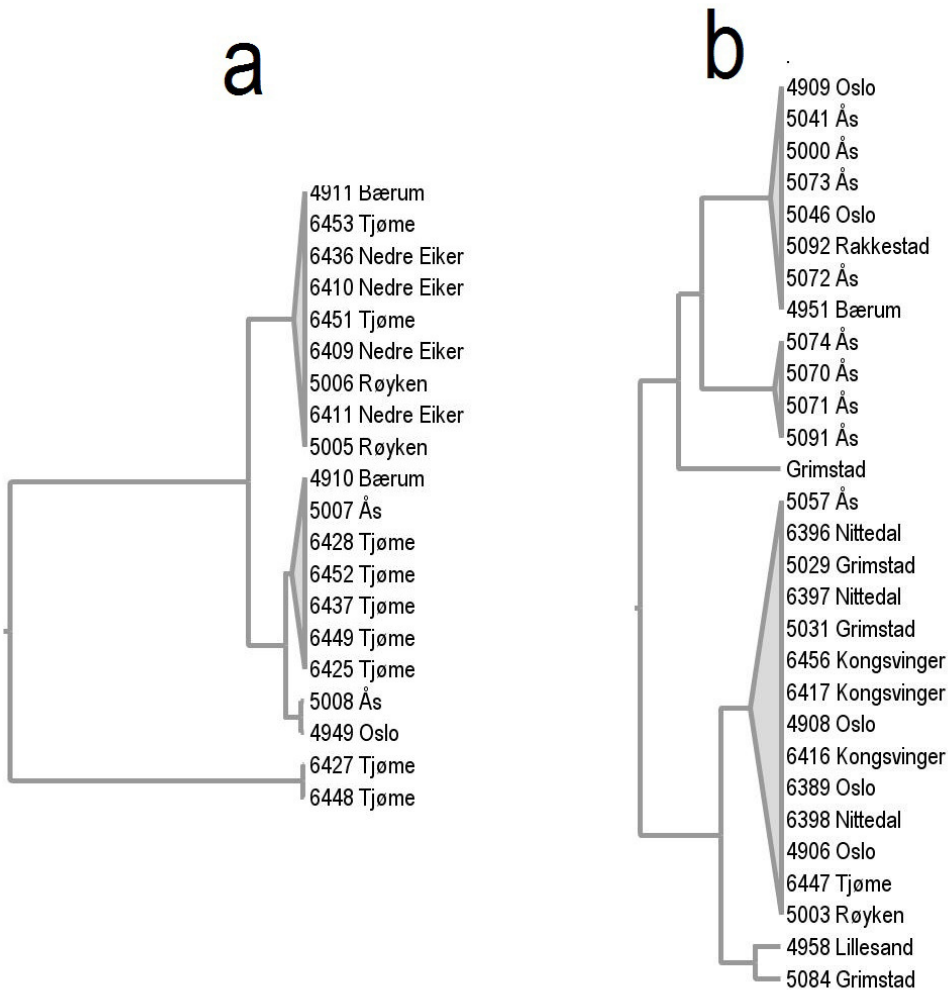

Figure 2 Clades of *Pteromalus elevatus* **(a)** and *Pteromalus albipennis* **(b)** from a Bayesian inference of the mitochondrial COI, where tips are labelled according to collecting site municipalities of the corresponding *Pteromalus* specimens. Note that there are no clear patterns in geographical occurrences of the specimens that can explain the intraspecific variation within the two clades.

Appendix 4

Table 1 Plant species of the family Asteraceae that were not found to be inhabited by members of *Pteromalus* or any fruit flies.

Species
Achillea millefololeum L.
Antennaria dioica (L.) Gaertn.
Antennaria alpina (L.) Gaertn.
Anthemis tinctoria L.
Carlina vulgaris L.
Erigeron borealis (Vierh.) Simmons
Hypochaeris maculata L.
Omalotheca sylvatica L.
Pilosella aurentiaca (L.) F.W.Schultz & Sch.Bip.
Senecio vulgaris L.
Solidago virgaurea L.
Sonchus asper (L.) Hill
Sonchus oleraceus L.
Tanacetum vulgare L.
Taraxacum F.H.Wigg
Tragopogon pratensis (Pers.) R.Bauer & Oberw
Tripleurospermum maritimum W.D.J.Koch