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Current state of knowledge on *Wolbachia* infection among Coleoptera: a systematic review

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Background. Despite great progress in studies on *Wolbachia* infection in insects, the knowledge about its relations with beetle species, populations and individuals, and the effects of bacteria on these hosts is still unsatisfactory. In this review we summarize the current state of knowledge about *Wolbachia* occurrence and interactions with Coleopteran hosts. **Methods.** An intensive search of the available literature resulted in the selection of 81 publications that describe the relevant details about *Wolbachia* presence among beetles. These publications were then examined with respect to the distribution and taxonomy of infected hosts and diversity of *Wolbachia* found in beetles. Sequences of *Wolbachia* genes (*16S rDNA, wsp* and *ftsZ*) were used for the phylogenetic analyses. **Results.** The collected publications revealed that *Wolbachia* has been confirmed in 197 beetle species and that the estimated average prevalence of this bacteria across beetle species is 38.3% and varies greatly across families and genera (0-88% infected members) and is much lower (c. 13%) in geographic studies. The majority of the examined and infected beetles were from Europe and East Asia. The most intensively studied have been two groups of herbivorous beetles: Curculionidae and Chrysomelidae. Coleoptera harbor *Wolbachia* belonging to three supergroups: F found in only 3 species, and A and B found in similar numbers of beetles (including some doubly infected); however the latter two were most prevalent in different families. 59% of species with precise data were found to be totally infected. Single infections were found in 69% of species and others were doubly- or multiply-infected. *Wolbachia* caused numerous effects on its beetle hosts, including selective sweep with host mtDNA (found in 3% of species), cytoplasmic incompatibility (detected in c. 6% of beetles) and other effects related to reproduction or development (like male-killing, possible parthenogenesis or haplodiploidy induction, and egg development). Phylogenetic reconstructions for *Wolbachia* genes rejected cospeciation between these bacteria and Coleoptera, with minor exceptions found in some Hydraenidae, Curculionidae and Chrysomelidae. In contrast, horizontal transmission of

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bacteria has been suspected or proven in numerous cases (e.g. among beetles sharing habitats and/or host plants). **Discussion.** The present knowledge about *Wolbachia* infection across beetle species and populations is very uneven. Even the basic data about infection status in species and frequency of infected species across genera and families is very superficial, as only c. 0.15% of all beetle species have been tested and/or examined so far. Future studies on *Wolbachia* diversity in Coleoptera should still be based on the Multi-locus Sequence Typing system and next-generation sequencing technologies will be important for uncovering *Wolbachia* relations with host evolution and ecology, as well as with other, co-occurring endosymbiotic bacteria.

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Abstract

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numerous effects on its beetle hosts, including selective sweep with host mtDNA (found in 3% of species), cytoplasmic incompatibility (detected in c. 6% of beetles) and other effects related to reproduction or development (like male-killing, possible parthenogenesis or haplodiploidy induction, and egg development). Phylogenetic reconstructions for *Wolbachia* genes rejected cospeciation between these bacteria and Coleoptera, with minor exceptions found in some Hydraenidae, Curculionidae and Chrysomelidae. In contrast, horizontal transmission of bacteria has been suspected or proven in numerous cases (e.g. among beetles sharing habitats and/or host plants).

Discussion. The present knowledge about *Wolbachia* infection across beetle species and populations is very uneven. Even the basic data about infection status in species and frequency of infected species across genera and families is very superficial, as only c. 0.15% of all beetle species have been tested and/or examined so far. Future studies on *Wolbachia* diversity in Coleoptera should still be based on the Multi-locus Sequence Typing system and next-generation sequencing technologies will be important for uncovering *Wolbachia* relations with host evolution and ecology, as well as with other, co-occurring endosymbiotic bacteria.

Key words: α-proteobacteria; beetles; evolution; ecology; endosymbiont; intracellular; interactions

Short title *Wolbachia* among Coleoptera: a review

Introduction

The relations between the intracellular α-proteobacterium *Wolbachia pipientis* Hertig 1936 (hereafter *Wolbachia*) and its hosts from various groups of arthropods and nematodes have been the object of much research and numerous publications (O’Neill et al., 1992; Werren et al., 1995a). The majority of these studies have focused on verifying endosymbiotic bacteria occurrence and diversity in various hosts at different levels: i) among selected species sharing a geographic area (e.g. O’Neill et al., 1992; Werren et al., 1995a, 2000), ii) among species inhabiting the same environment or that are ecologically-associated (e.g. Stahlhut et al., 2010), iii) among species from particular taxonomic groups (e.g. Czarnetzki et al., 2004; Lachowska et al., 2010; Sontowski et al., 2015), and iv) within populations of selected taxa (e.g. Stenberg et al., 2004; Mazur et al., 2016). Another branch of research on the relations between *Wolbachia* and its hosts has focused on host species phylogenetics or population genetics, which is in some cases related to population differentiation and speciation (e.g. Kubisz et al., 2012; Montagna et al., 2014). In this research *Wolbachia* is sometimes treated as an additional "marker" – a source of genetic data about the eco-evolutionary relations of its hosts. A third type of *Wolbachia* studies has concerned the direct or indirect effects of the infection on host fitness, development or survival at the individual and population levels (e.g. Weeks 2002; O'Neill 2007). Moreover, in a separate branch of research (or in conjunction with the abovementioned types of studies), *Wolbachia* is often examined directly, mainly with respect to strain diversity, distribution and relations with other strains or different co-existing bacteria (Baldo et al., 2007). All these branches of research have substantially extended the knowledge about the relations between the most widespread intracellular endosymbiont – *Wolbachia* and its various hosts. Moreover, these studies have been expanded to encompass other bacteria with similar biologies and effects on hosts (like *Cardinium, Spiroplasma, Rickettsia*) (Zchori-Fein & Perlman 2004; Goto et al., 2006; Duron et al., 2008); however, a great majority of studies are still conducted on *Wolbachia* (Zug et al., 2012). Recently, the various *Wolbachia* supergroups have been proposed to belong to several "*Candidatus* Wolbachia" species (Ramírez-Puebla et al., 2015); however, this approach has been criticized (Lindsey et al., 2016). Due to the uncertain species status of the "*Candidatus* Wolbachia" and because all previous studies considered these presumed different species as distant supergroups, in this review we have followed the previous *Wolbachia* taxonomy.

In summary, *Wolbachia* has been detected in 10-70% of examined hosts (Jeyaprakash & Hoy 2000; Hilgenboecker et al., 2008), depending on the geographical, ecological or taxonomical association of the selected species. Moreover, more detailed studies, at the population level, have shown that infection is not as straightforward as was assumed in the early stages of *Wolbachia* research. More and more species have been found to be only partially infected, e.g. in only some parts of their ranges or infection was associated with only some phylogenetic lineages (usually correlated with the distribution of mitochondrial lineages) (Clark et al., 2001; Roehrdanz et al., 2006). Furthermore, examples of multiply infected species and individuals have been reported, which has important consequences for the understanding of some of the effects of *Wolbachia* infection (Malloch et al.,. 2000; Gurfield, 2016). *Wolbachia* is known to have numerous effects on its hosts, among which the most interesting and important are those that disturb host reproduction, such as cytoplasmic incompatibility, thelytokous parthenogenesis, feminization of genetic males, male-killing, increased mating success of infected males via sperm competition and the host's complete dependence on bacteria for egg production (for reviews see Werren, 1997; Werren & O'Neill, 1997 and Stouthamer et al., 1999). Some of these effects are responsible for diversification of host populations and consequently for speciation (e.g. by the selective sweep of mtDNA or the whole genome of the infected host with the genome of bacteria; Keller et al., 2004; Mazur et al., 2016). This could be another major factor, additional to those already known, responsible for radiation of insects and particularly beetles.

There are several reviews summarizing the state of knowledge on *Wolbachia* infection among various taxonomic groups of nematodes and arthropods. Over the last years, such reviews have been prepared for the following groups: filarial nematodes (Filarioidea) (Taylor & Hoerauf, 1999; Casiraghi et al., 2001), crustaceans (Crustacea) (Cordaux et al., 2001), spiders (Araneae) (Goodacre et al., 2006; Yun et al., 2010), mites (Acari) (Chasirini et al., 2015), springtails (Collembola) (Czarnetzki et al., 2004), Heteropteran Bugs (Heteroptera) (Kikuchi et al., 2003), ants (Formicidae) (Russell, 2012), wasps (Hymenoptera: Apocrita) (Schoemaker et al., 2002) and butterflies (Lepidoptera) (Tagami et al., 2004). Surprisingly, there is no such review for beetles (Coleoptera), which are the most species rich and diversified group of organisms on Earth, which are known from most habitats, and whose members belong to all major trophic guilds of animals. Some groups of beetles have been examined with respect to *Wolbachia* infection, but usually only with a limited coverage of species (e.g. weevils, Curculionidae, Lachowska et al., 2010; leaf beetles; Chrysomelidae, Clark et al., 2001, Jäckel et al., 2013; jewel beetles; Buprestidae, Sontowski et al., 2015 and minute moss beetles, Hydraenidae, Sontowski et al., 2015).

In this review we have summarized the current state of knowledge on the relations between beetles and *Wolbachia* by referring to all the abovementioned groups of research.

Moreover, we have highlighted future research directions concerning *Wolbachia* relationships with their diverse Coleopteran hosts.

Survey Methodology

We searched the scientific literature with Web of Knowledge databases, using the following combination of keywords linked by AND (the Boolean search term to stipulate that the record should contain this AND the next term): "*Wolbachia*" AND "Coleoptera" and "*Wolbachia*" AND "beetles". Our final literature search for this analysis was conducted on December 22, 2017. This produced 322 results. Each result was inspected to determine whether or not it contained information on the subject matter. Articles that had no relevance (e.g. any reports that were not about *Wolbachia*-Coleoptera relations, including those that only had some references to either beetles or bacteria in the citations) were excluded. After the removal of duplicates, 65 were excluded from the remaining articles (n = 234) for not being direct reports about *Wolbachia*-Coleoptera relations, 44 were excluded because they examined other hosts and only referred to publications on Coleoptera, and 44 others were excluded because they referred to data already presented in previous publications on Coleoptera. The use of two alternative and comprehensive scientific collections should have reduced any biases. Each document was read critically for the information that it contained on *Wolbachia*-Coleoptera relations, with special reference to answering the study questions listed below. Figure 1 shows a flow diagram for the systematic review following Prisma guidelines (Moher et al., 2009).

We examined the collected data on various aspects of *Wolbachia* infection in Coleoptera with respect to the following: the i) characteristics of the publications (to determine the scope and progress of studies on *Wolbachia*) (n=81), ii) geographic distribution of infected beetle species and populations (n=79), iii) sampling design (how many sites and individuals were examined) (n=63), iv) characteristics of the markers (genes) used for genotyping the bacteria (n=77) and their hosts (n=34), v) numbers and frequencies of species found to be infected in particular beetle families and genera (n=58), vi) supergroup prevalence in examined taxonomic groups (n=40), vii) strain distribution and diversity in populations and individuals (n=30), vii) effects of *Wolbachia* on its beetle hosts (n=39). Statistical analyses were done in Statistica 11 (Statsoft).

Finally, we downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and the *Wolbachia* MLST database (https://pubmlst.org/wolbachia/) all available sequences of *Wolbachia* genes found in any species of beetle. We restricted further analyses to the most widely used bacteria genes, i.e. *16S rDNA, Wolbachia* surface protein gene *wsp* and cell division protein gene *ftsZ*. Because of the different lengths and spans of available sequences, the long parts of the 3' and 5' ends of each gene were trimmed, which resulted in alignments of length 663 bp for *16S rDNA*, 355 bp for *wsp* and 241 bp for *ftsZ*. The length of the *ftsZ* alignment was particularly short as two different sets of primers have been used for its amplification, and its amplicons only overlapped across a relatively short part of the gene. Phylogenetic trees were only reconstructed for unique gene variants found in particular host taxa. Trees were inferred using Maximum Likelihood (ML) implemented in IQ-TREE web server http://www.iqtree.org/ (Trifinopoulos et al., 2016) under the following settings Auto selection of substitution model, ultrafast bootstrap approximation (UFBoot) (Minh et al., 2013) with 10000 iterations, maximum correlation coefficient = 0.99, single branch test with use of the approximate Likelihood-Ratio Test (SH-aLRT) (Anisimova & Gascuel, 2006; Guindon et al., 2010) and other default options.

The nomenclature of host taxa and their systematic positions throughout the paper follow the articles from which the data was derived.

Characterization of *Wolbachia* infection among Coleoptera

Publications

The final list of publications concerning data about *Wolbachia* infection in Coleoptera comprised 81 papers (Supplementary Table 1). The oldest articles with relevant information about *Wolbachia* infection in beetles were published in 1992 (Campbell et al., 1992; O'Neill et al., 1992), and the number of articles since then has increased significantly year by year (Spearman correlation = 0.817; Fig. 2). The majority of these articles (63%) concerned infection in only single beetle species, whereas 20% discussed infection in multiple species belonging to the same genus, 6% − multiple species from the same family, 6% − various species of Coleoptera et al., and a further 5% − studies on geographic groups of insects that included some, usually random species of beetles (O'Neill et al., 1992; Werren et al., 1995, 2000).

Most studies were done on Curculionidae (33) and Chrysomelidae (31), following Tenebrionidae (9), Coccinellidae (7) and Sylvanidae (3) (Supplementary Table 1). The members of all other families were investigated in only 1-2 studies. Consequently, 2.5 and 1.6 Curculionidae and Chrysomelidae species were respectively examined per article. All species of Hydraenidae and Buprestidae were included in only single articles (Sontowski et al., 2015), whereas limited numbers of species of Coccinellidae and Tenebrionidae were examined in several articles (Hurst et al., 1999; Fialho & Stevens 1996, 1997, 2000; Majerus et al., 2000; et al., Weinert et al., 2007; Elnagdy et al., 2013; Ming et al., 2015; Goodacre et al., 2015; Kageyama et al., 2015; Li et al., 2015; Li et al., 2016; Dudek et al.; 2017). *Wolbachia* infection was only studied more than once in 20 species.

Sampling design

The majority of species investigated with respect to *Wolbachia* infection were from Europe, and a relatively high number of species were from Asia and both Americas, whereas only ten infected species were from Africa, and three from Australia-Oceania (Fig. 3). A number of publications describing *Wolbachia* infection in Coleoptera had similar geographic coverages (Fig. 3).

Studies were done on samples collected from an average of 5.2 sites and concerned on average 53.0 specimens, or if excluding the most widely studied families Curculionidae and Chrysomelidae, 6.0 sites and 65.1 individuals (Fig. 4). For Curculionidae and Chrysomelidae, these numbers were on average 4.4 and 6.0 sites, respectively, and 40.7 and 70.2 individuals, respectively (Fig. 4). The numbers of sites and individuals examined in particular groups were insignificantly different, with the exception of the numbers of examined individuals in Curculionidae and Chrysomelidae (Fig. 4).

Examined genetic markers

The most often used *Wolbachia* gene for studies on Coleoptera was *ftsZ*, followed by *hcpA, wsp* and *16S rDNA* (Fig. 5). Most studies using *hcpA* also used other MLST genes, including *ftsZ.* On the other hand, many species were only investigated with either *16S rDNA* or *wsp* or *ftsZ* alone. Single studies used *groEL* (*Monochamus alternatus*, Aikawa et al., 2009; *Tribolium madens*, Fialho & Stevens, 2000) or *ITS* genes (*Tribolium madens*, Fialho & Stevens, 2000). So far, only five studies have used next-generation sequencing technology (Illumina or 454) to detect *Wolbachia*; two used *16S rDNA* for metabarcoding of microbiota (*Sitona obsoletus*, *Steriphus variabilis*, White et al., 2015; *Aleochara bilineata* and *Aleochara* bipustulata, Bili et al., 2016; *Hylobius abietis*, Berasategui et al., 2016; *Brontispa longissimi*, Takano et al., 2017; *Harmonia axyridis*, Dudek et al., 2017) and one used shotgun genomic sequencing (*Amara alpine,* Heintzman et al., 2014). For genotyping of hosts, 52.4% of studies utilized fragments of *COI* from mtDNA (usually a barcode fragment of this gene). Fewer studies (23.1%) analyzed *rDNA* (usually *ITS1* and/or *ITS2* spacers), *EF1α* (14.0%), Wingless (2.2%), Histone H3 (2.2%) and microsatellites (6.1%). In *Wolbachia*-related studies, host genes have been used for several purposes like i) using host DNA as a control for genetic material quality, ii) barcoding for host species identification, iii) phylogenetics, phylogeography and population genetics, iv) estimating co-evolutionary relations between the bacteria and host, and v) detecting some of the effects of *Wolbachia* on its hosts (like linkage disequilibrium, selective sweep, cytoplasmic incompatibility).

Taxonomic coverage

The beetles examined with respect to *Wolbachia* infection belong to 22 families (Micromalthidae, Gyrinidae, Haliplidae, Noteridae, Dytiscidae, Carabidae, Staphyllinidae, Hydrophilidae, Hydraenidae, Anobiidae, Dermestidae, Buprestidae, Byturidae, Cleridae, Lampyridae, Coccinellidae, Tenebrionidae, Meloidae, Sylvanidae, Cerambycidae, Chrysomelidae, Curculionidae). In total 197 beetle species were found to harbor *Wolbachia* infection; however the distribution of infected species among families varied markedly. The highest numbers of infected beetle species were found for the Curculionidae (79 species), Chrysomelidae (51 species), Hydraenidae (14 species), Buprestidae (13 species), Coccinellidae (9 species) and Dytiscidae (8 species) (Fig. 6). In all other families only 1-3 species were reported to harbor *Wolbachia* (Supplementary Table 1). However, these numbers are biased by the low number of articles (studies) dealing with members of particular beetle families (see above).

Considering infection across beetle genera, the most richly infected genera were *Altica* (Chrysomelidae, 17 species), *Naupactus* (Curculionidae, 11 species), *Hydraena* (Hydraenidae, 8 species) and *Agrilus* (Buprestidae, 6 species) (Supplementary Table 1). In total, 49 genera were found to have infected members (Supplementary Table 1, Table 1). The infection in Coleoptera was estimated at 38.3% of examined species; however, the proportion of infected species varied greatly between families and genera. At the family level the infection frequency was from 10.5% (Tenebrionidae) to 100% (Noteridae) (Goodacre et al., 2015, Sontowski et al., 2015); however when considering only families for which more than 30 species were investigated (e.g. Clark et al., 2001; Lachowska-Cierlik et al.; 2010, Rodriguer et al.; 2010a, Kondo et al.; 2011, Jäckel et al., 2013; Sontowski et al.; 2015, Kawasaki et al., 2016), infection was found in up to 63% of species (Hydraenidae) (Table 1). At lower taxonomic levels, *Wolbachia* was found in 25% of Diabroticite (Chrysomelidae; Clark et al., 2001), 14.3-16.7% of Bruchina (Chrysomelidae; Kondo et al., 2011), 34.8% of Scolytinae (Curculionidae, Kawasaki et al., 2016) and 16.7% of Curculioninii (Toju et al., 2013). Among 54 genera in which *Wolbachia* infection was examined for at least 2 species, 12 genera were completely uninfected, while 6 genera were completely infected (Table 1). If considering only genera with at least 5 verified species, *Wolbachia* was found in 0% (*Acmaeodera*; Buprestidae; Sontowski et al., 2015) to 88% species (*Altica*, Chrysomelidae; Jäckel et al., 2013). There was only a marginally negative and insignificant correlation between the number of examined and number of infected species (R=-0.078). If considering only the most widely examined families: Chrysomelidae and Curculionidae, the difference in infection frequency between these two groups was insignificant (Z=-1.656, P=0.098). Geographic studies on *Wolbachia* prevalence in insects have found much lower frequencies of infection in Coleoptera species: the bacterium was found in only 10.5% of beetles from Panama and 13.5% of beetles from North America (Werren et al., 1995a, 2000).

Wolbachia diversity

Among the various beetle species, *Wolbachia* strains belonged to three supergroups (A, B and F). However, they occurred at very different proportions in different groups of beetles, and these differences were significant (Chi^2=98.78, P=0.000). Overall, the proportion of beetle species found to be infected with *Wolbachia* strains belonging to supergroups A or B was similar, with approx. 12% of all species harboring either supergroup (either as single infections in different species or populations or as multiple infections within individuals) (Fig. 7), whereas supergroup F was found in only 3 beetle species: *Agrilus araxenus* and *Lamprodila mirifica* (both Buprestidae; Sontowski et al., 2015) and *Rhinocyllus conicus* (Curculionidae; Campbell et al., 1992). In the four groups of beetles with the highest numbers of examined and infected species, the distributions of supergroups varied: in Buprestidae, a similar numbers of species were infected by supergroups A and B (all singly infected), with a relatively high proportion of F infected species (Sontowski et al., 2015). In contrast, in Hydraenida, supergroup A dominated over supergroup B (Sontowski et al., 2015). This was also the case in Chrysomelidae, with some species infected by both strains (Kondo et al., 2011,; Jäckel et al., 2013; Kolasa et al., 2017). The most varied infections were observed in Curculionidae, with supergroup B dominating, a presence of taxa infected by both A and B supergroups, and a single species infected by F supergroup (Lachowska-Cierlik et al.; 2010, Rodriguer et al., 2010a; Kawasaki et al., 2016) (Fig. 7). Considering the frequency of infected specimens in the examined beetle species represented by the available data (N=106), 63 species were reported to be totally infected (all individuals possessed *Wolbachia*), whereas 43 species had this bacterium in only some individuals (if exclude Chrysomelidae and Curculionidae: 8 and 15 species, respectively) (Fig. 8). The same calculated for Chrysomelidae resulted in 17 and 10 species, respectively, and for Curculionidae in 38 and 18 species, respectively (Fig. 8). These differences between these values (between these groups of species) were significant (Chi^2=72.03, P=0.000). A single *Wolbachia* strain was observed in 43 species (species with available data N = 62), whereas two strains were reported in 10 species (*Byturus tomentosus*,Malloch et al., 2000; *Altica quercetorum*, Jäckel et al., 2013; *Callosobruchus chinensis,* Okayama et al., 2016; *Chelymorpha alternans,* Keller et al., 2004; *Crioceris quaterdecimpunctata* and *Crioceris quinquepunctata*, Kolasa et al., 2017; *Adalia bipunctata*, Majerus et al., 2000; *Polydrusus inustus*, Kajtoch et al., 2012; *Cyanapion afer* and *C. spencii,* Kajtoch et al., 2017) and multiple infection in a further 9 species (*Callosobruchus chinensis*, Kondo et al., 2002; *Diabrotica barberi,* Roehrdanz & Levine, 2007; *Conotrachelus nenuphar*, Zhang et al., 2010; *Pityogenes chalcographus*, Arthofer et al., 2009; *Xyleborus dispar* and *Xylosandrus germanus*, Kawasaki et al., 2016) (Fig. 8). In Chrysomelidae (N=22) these numbers were 12, 5 and 5, respectively and in Curculionidae (N=37), 30, 3 and 4, respectively (Fig. 8). The numbers of single, double and multiple infected individuals in these groups of beetles differed insignificantly (Chi^2 ANOVA=2.364, P=0.307).

Effects on hosts

Wolbachia affected beetle hosts in several ways. Linkage disequilibrium and/or selective sweep between bacteria and host genomes (usually with host mtDNA) were detected in 6 species (3% or 9% if excluding Chrysomelidae and Curculionidae): 2 (4%) Chrysomelidae (*Altica lythri,* Jäckel et al., 2013; *Aphthona nigriscutis*, Roehrdanz et al., 2006) and 4 (5%) Curculionidae (*Eusomus ovulum,* Mazur et al., 2016; *Naupactus cervinus,* Rodriguero et al., 2010b, *Polydrusus inustus, Polydrusus pilifer*, Kajtoch et al., 2012). Cytoplasmic incompatibility was detected or suspected but unconfirmed in 12 (6% or 18% if excluding Chrysomelidae and Curculionidae) Coleoptera: 6 (13%) Chrysomelidae (*Chelymorpha alternans,* Keller et al., 2004*, Diabrotica barberi,* Roehrdanz & Levine 2007, et al.,*Diabrotica virgifera virgifera*, Giordano et al., 1997; *Callosobruchus chinensis*, Kondo et al., 2002; *Callosobruchus analis*, Numajiri et al., 2017; *Brontispa longissimi*, Takano et al., 2017), 3 (4 %) of Curculionidae (*Cossomus sp.,* Zhang et al., 2010*; Hypothenemus hampei,*Mariño et al., 2017, *Xylosandrus germanus*, Kawasaki et al., 2016), 1 of Sylvanidae (*Oryzaephilus surinamensis*, Sharaf et al., 2010) and 1 of Tenebrionidae (*Tribolium confusum*, Li et al., 2016, Ming et al., 2015). Horizontal transfer of *Wolbachia* was detected or suspected in 26 species of Coleoptera (13% or 39% if excluding Chrysomelidae and Curculionidae) − 16 (33%) species of Chrysomelidae (several species of *Altica,* Jäckel et al., 2013, *Crioceris quaterdecimpunctata* and *Crioceris quinquepunctata*, Kolasa et al., 2017) and 10 (14%) species of Curculionidae (members of *Euwallacea,Xyleborus, Xylosandrus*, *Xyleborinus schaufussi* and *Taphrorychus bicolor*, Kawasaki et al., 2016, *Polydrusus* and *Parafoucartia squamulata,* Kajtoch et al., 2012; *Sitophilus oryzae* and *S. zaemais*, Carvalho et al., 2014). Other effects of *Wolbachia* on beetles included the following: i) transfer of bacteria genes to the autosomes of the host (so far detected only for *Monochamus alternatus*, Cerambycidae, Aikawa et al., 2009 and *Callosobruchus chinensis*, Chrysomelidae, Nikoh et al., 2008); ii) coexistence of *Wolbachia* with *Rickettsia* (*Calvia quattuordecimguttata, Coccidula rufa, Coccinella septempunctata, Halyzia sedecimguttata, Rhizobius litura*, Weinert et al., 2007; *Sitona obsoletus*, White et al., 2015; *Micromalthus debilis*, Perotti et al., 2016) in the host or with *Spiroplasma* (*Chilocorus bipustulatus*, Weinert et al., 2007; *Aleochara bipustulata*, Bili et al., 2016) or with both (*Adalia bipunctata,* Majerus et al., 2000, *Harmonia axyridis*, Dudek et al., 2017; both Chrysomelidae; *Curculio sikkimensis*, Toju & Fukatsu, 2011; *Aleochara bilineata*, Bili et al., 2016); iii) induction and reinforcement of parthenogenesis, however this effect had weak support and had other possible alternative explanations (numerous species of Naupactini, Rodriguer et al., 2010a and *Eusomus ovulum*, Mazur et al., 2016; all Curculionidae; *Micromalthus debilis*, Perotti et al., 2016); iv) possible induction of haplodiploidy (*Euwallacea interjectus, Euwallacea validus*, Curculionidae, Kawasaki et al., 2016); v) male-killing (*Tribolium madens*, Tenebrionidae, Fialho & Stevens, 2000); vi) necessity of infection for egg development (*Otiorhynchus sulcatus*, Curculionidae, Son et al., 2008; *Coccotrypes dactyliperda*, Zchori-Fein et al., 2006); vii) populations evolving towards endosymbiont loss and repeated intraspecific horizontal transfer of *Wolbachia* (*Pityogenes chalcographus,* Curculionidae, Arthofer et al., 2009), viii) fitness decline in infected beetles (*Callosobruchus analis*, Numajiri et al., 2017), ix) modification of sperm (*Chelymorpha alternans*, Clark et al., 2008), x) down-regulation of defense genes in host plants (maize in *Diabrotica virgifera virgifera*, Barr et al., 2010).

Phylogenetic relations

The tree reconstructed for *16S rDNA* included 52 sequences from bacteria found in 45 host beetle species. This tree included three major lineages, with separate clusters of *Wolbachia* sequences belonging to A, B and F supergroups (Supplementary Fig. 1). F supergroup was represented by a single sequence from *Rhinocyllus conicus* (Curculionidae) (Supplementary Fig. 1). Sequences assigned to supergroup A (based on information available in the articles) were found to be polyphyletic. Some *16S* sequences from *Xylosandrus* spp. and *Curculio* spp. (Curculionidae), or *Oreina cacaliae* and *Galeruca tanaceti* (Chrysomelidae) clustered as a sister lineage to all other A and B sequences (Supplementary Fig. 1). Overall, the diversity of *16S* sequences assigned to supergroup B was much greater than those assigned to supergroup A (Supplementary Fig. 1).

The tree reconstructed for *ftsZ* included 131 sequences found in 114 host beetle species. The *ftsZ* phylogenetic tree resulted in a topology similar to that of *16S rDNA* − it included groups of sequences belonging to A, B and F supergroups (Supplementary Fig. 2). Supergroup F was represented by *Agrilus araxenus* and *Sphaerobothris aghababiani* (both Buprestidae). Moreover, the supergroup B clade was divided into two clusters, among which one included a small group of sequences found in four beetle hosts: *Chelymorpha alternans* (Chrysomelidae)*, Eurymetopus fallax, Sitophilus oryzae* and *Conotrachelus nenuphar* (all three Curculionidae) (Supplementary Fig. 2). Also in this gene, the genetic variation of sequences belonging to supergroup A was much lower, and only a few sequences were highly diverged (e.g. strains of *Callosobruchus* *chinensis*, Chrysomelidae; *Tribolium confusum*, Tenebrionidae or *Polydrosus pilosus,* Curculionidae) (Supplementary Fig. 2). There was also one slightly distinct clade that mainly consisted of bacteria sequences found in some Hydraenidae, Curculionidae and Chrysomelidae (Supplementary Fig. 2).

The *Wsp* tree was built for 100 sequences found in 82 hosts. This network resulted in two clusters representing supergroups A and B; among the available sequences there were no representatives of supergroup F (Supplementary Fig. 3). *Wsp* was found to be more diverse than *16S* and *ftsZ*, as it had multiple distant lineages in both supergroups. Within supergroup B the most distant lineage originated from the only *wsp* sequence found in *Callosobruchus analis* (Chrysomelidae) (Supplementary Fig. 3). In this supergroup, two distinct clades could also be delineated: one consisting of *Wolbachia* sequences found in a variety of beetle hosts and the second mainly consisting of hosts from Curculionidae (*Otiorhynchus singularis, Sitophilus* spp.), Chrysomelidae (*Callosobruchus* spp., *Acromis sparsa*) and Byturidae (*Byturus tomentosus*) (Supplementary Fig. 3). Similarly, in supergroup A several distinct lineages could be delineated, consisting of *Wolbachia* sequences found in e.g. *Ceutorhynchus obstrictus* (Curculionidae), *Diabrotica* spp., *Oreina* spp. and *Aphthona* spp. (all Chrysomelidae) – which are all represented by several strains (Supplementary Fig. 3).

The abovementioned phylogenetic reconstructions of the relations among *Wolbachia* strains identified on the basis of polymorphism of several genes show that there is no strict correlation between host phylogeny and bacterial strain relationships. Even in studies that covered multiple related species (e.g. those belonging to the same genus), evidence for direct inheritance of *Wolbachia* strains from common ancestors is restricted to Hydraenidae (Sontowski et al., 2015) and some species of *Oreina* (Montagna et al., 2014) or *Curculio* (Toju et al., 2013). In the case of *Altica,* the data show that cospeciation was rare and restricted to a few recently diverged species (Jäckel et al., 2013). In contrast, there are numerous examples of phylogenetically related beetle species possessing different *Wolbachia* strains (e.g. Lachowska et al., 2010). It is also often the case among related species that some are infected, whereas others not (*Crioceris*, Kubisz et al., 2012; *Oreina,* Montagna et al., 2014; *Cyanapion*, Kajtoch et al., 2017); so any assumption that the bacteria was inherited from a common ancestor would also need to consider multiple losses of infection. The latter phenomenon is probable; however, there is no direct evidence from natural populations, at least in studies on beetles, of *Wolbachia* disappearing over time. Some exemplary studies that found *Wolbachia* present in related species, after detailed examination, rejected the idea that bacteria was inherited from a common ancestor. This was because different host species harbored unrelated stains (e.g. among weevils, Lachowska et al., 2010, Rodriguer et al., 2010a) or in cases where strains were identical or similar, the hosts were not phylogenetically close to each other (e.g. *Crioceris*, Kubisz et al., 2012). Finally, there is evermore proof of horizontal *Wolbachia* transmission via different mechanisms, such as via predators, parasitoids, common habitat or foraging on the same host plants (Huigens et al., 2004; Stahlhut et al., 2010; Caspi-Fluger et al., 2012; Ahmed et al., 2015; Kolasa et al., 2017). Studies on beetles have mainly provided indirect evidence of such transmissions. There are known groups of species that inhabit the same environments and share the same or very similar *Wolbachia* strains, e.g. steppic weevils from East-central Europe (Mazur et al., 2014) and bark beetles in Japane (Kawasaki et al., 2016). Recently, evidence for has also appeared for the role of host plants in bacteria spread – *Wolbachia* DNA was detected in two species of *Crioceris* leaf beetles and in their host plant – *Asparagus* spp. (Kolasa et al., 2017).

Finally, in light of the proposed "*Candidatus* Wolbachia" species, the summarized phylogenetic relations among *Wolbachia* strains infecting various beetles indicate that the taxonomic distinctiveness of supergroups is inconclusive (Ramírez-Puebla et al., 2015; Lindsey et al., 2016). First, beetles generally harbor members of supergroups A and B, and only occasionally members of supergroup F. Therefore, it is not possible to make any conclusions about broader *Wolbachia* taxonomy based only on *Wolbachia* strains found in Coleoptera. However, there are numerous examples of beetle hosts harboring both supergroups, including beetles in which some *Wolbachia* genes are of supergroup A origin, while others are of supergroup B origin; this indicates that recombination between strains belonging to different supergroups is quite frequent. This is evidence against the designation of the "*Candidatus* Wolbachia" species, at least with respect to members of supergroup A and B.

Current gaps and future endeavors

The present knowledge on *Wolbachia* infection across beetle species and populations is very uneven. Even the basic data about infection statuses in species and frequencies of infected species across genera and families is superficial, as there are only c. 200 beetle species known to be infected. This means that if 38% is the average frequency of infection among beetle species, then only c. 530 species have been tested so far. This is merely c. 0.15% of the total number of beetles, which is estimated to be around 360 000 species (Farrell, 1998; Bouchard et al., 2009). We know even less at the population level, as the majority of beetle species have only had single individuals tested for *Wolbachia* infection (e.g. Lachowska et al., 2010, Sontowski et al., 2015). These very basic screens have probably underestimated the number of infected species because of false-negative results obtained for species with low or local infection in populations. On the other hand, these preliminary estimates could have overestimated the real number infected beetles, as sampling in these studies was rarely random and most often focused on specific groups, e.g. on genera for which preliminary data suggested the presence of *Wolbachia* infection. Indeed, an intensive search of *Wolbachia* infection across hundreds of beetle species from Europe suggested a lower infection rate – c. 27% to be infected (Kajtoch et al., unpublished). Also, knowledge about infection at the geographic scale is very uneven, and only Europe and Asia (basically China and Japan) have been relatively well investigated. There is a huge gap in the knowledge for African, Australian and Oceanian beetles, where a high diversity of beetles exists and probably a similar diversity of *Wolbachia* could be expected (e.g. compared to preliminary data available from Central and South America (Werren et al., 1995; Rodriguer et al., 2010a)).

Little is known about *Wolbachia* diversity in beetle hosts, as the majority of studies used only single genetic markers, and often different genes were sequenced for different taxa. This precludes complex analysis of *Wolbachia* diversity across all tested beetle hosts. This has changed since 2006, since Baldo et al., (2006) proposed Multilocus Sequence Typing (MLST), which is based on the genotyping of five housekeeping genes, usually in conjunction with *wsp* sequencing. MLST is and should remain a sufficient way to understand basic *Wolbachia* diversity. On the other hand, to fully understand *Wolbachia* relations among strains and supergroups (or presumed species), between *Wolbachia* and its hosts and especially between *Wolbachia* and other microorganisms, amplicon-sequencing (e.g. *16S* rDNA) or genome-sequencing are needed. This could be achieved thanks to the development of next-generation sequencing technologies (NGS). Surprisingly, despite fast development of NGS in the last years, very few studies have used this technology for studying *Wolbachia* in beetle populations. For example, five studies sequenced *16S* amplicons generated from microbiota and detected

Wolbachia (White et al., 2015; Bili et al., 2016; Berasategui et al., 2016; Takano et al., 2017; Dudek et al., 2017). The only single study that utilized shotgun sequencing was executed for other purposes and also accidentally showed *Wolbachia* genes in examined species (Heintzman et al., 2014). NGS is probably the best prospect for studies on *Wolbachia* infection and diversity, and will help to answer most current riddles and issues.

The big challenge is to understand the impact of infection on beetle biology, physiology and ecology. It is known that *Wolbachia* has several effects on host reproduction, but relatively few studies prove or suggest e.g. cytoplasmic incompatibility, male-killing or other effects on the development of selected beetles (Clark et al., 2001; Keller et al., 2004 Roehrdanz et al., 2006 Roehrdanz & Levine 2007 Sharaf et al., 2010 Zhang et al., 2010; Jäckel et al., 2013; Ming et al., 2015; Kawasaki et al., 2016; Li et al., 2016; Mariño et al., 2017; Numajiri et al., 2017; Takano et al., 2017). It is very probable that this bacteria has large and frequent effects on beetle reproduction and is consequently partially responsible for beetle radiation, at least in some taxonomic groups, geographic areas or habitats. Also very few studies have shown data on linkage disequilibrium and selective sweep between bacteria and host genomes (Roehrdanz et al., 2006*;* Rodriguero et al., 2010b; Kajtoch et al., 2012; Jäckel et al., 2013; et al. Mazur et al., 2016). These effects could also have led to the speciation of numerous beetles. Moreover, this phenomenon could have serious implications for beetle barcoding, as selective sweep is known to reduce mitochondrial diversity in its hosts and therefore could decrease the number of identified species (Hurst & Jiggins, 2005). On the other hand, cytoplasmic incompatibility can lead to the origin of highly diverged phylogenetic mitochondrial lineages within species, which would increase the number of identified taxa (Smith et al., 2012). Also here, NGS technologies will enable more sophisticated analyses of these genetic relations and their effects (e.g. by the sequencing of transcriptomes for physiological studies or by genotyping-by-sequencing for phylogenetic studies). Genotyping with NGS should also verify whether the recent assumption that different supergroups are indeed "*Candidatus* Wolbachia" species is correct or not (Ramírez-Puebla et al., 2015; Lindsey et al., 2016).

Only very preliminary results suggested *Wolbachia* was not only transmitted vertically, but that it could also have spread horizontally (Jäckel et al., 2013; Carvalho et al., 2014; Kawasaki et al., 2016; Kolasa et al., 2017; Mazur et al., 2017). In light of the general lack of cospeciation between bacteria and beetles, horizontal transmission must be a highly underestimated phenomenon. Horizontal transmission of *Wolbachia* among beetles, cannot be confirmed without considering other coexisting insects that can mediate transmission, such as predators, parasitoids or beetle prey. Moreover, other arthropods that share habitats with beetles, e.g. phoretic ticks (Hartelt et al., 2004) and nematodes (Casiraghi et al., 2001), need to be examined. Finally, host plants are promising objects of studies on *Wolbachia* transmission across beetle populations (Kolasa et al., 2017), as phloem is probably an important mediator of this bacteria's spread across insect populations (DeLay, 2012; Li et al., 2016). Concerning transmission – another very interesting topic is the transfer of *Wolbachia* genes into host genomes (Dunning Hotopp et al., 2007; Koutsovoulos et al., 2014; Funkhouser-Jones et al., 2015). This issue has only been reported twice for beetle hosts so far (Nikoh et al., 2008; Aikawa et al., 2009). This problem could be important as if such transfers are frequent, simple testing of *Wolbachia* presence in a host based on single or even several gene sequencing could overestimate the number of truly infected species, populations or individuals.

Finally, a very interesting topic for future studies is the examination of the presence of other intracellular and symbiotic bacteria (like *Cardinium, Spiroplasma, Rickettsia*), in Coleoptera and their relations, both with the host and *Wolbachia*. So far, only three studies have found *Wolbachia* with *Rickettsia* and/or *Spiroplasma* together in beetle hosts (Majerus et al., 2000; Weinert et al., 2007; Toju & Fukatsu 2011; White et al., 2015; Perotti et al., 2016; Bili et al., 2016; Dudek et al., 2017). Preliminary results suggest that there is some balance in the number of these bacteria, probably caused by competition within host cells (Goto et al., 2006). A recent summary of the presence of these bacteria in insects showed that *Rickettsia* has been found in single species of Micromalthidae, Staphylinidae, Buprestidae, Coccinellidae and Curculionidae (Werren et al., 1994; Lawson et al., 2001; Weinert et al., 2007; Toju & Fukatsu 2011; White et al., 2015; Perotti et al., 2016; Bili et al., 2016), *Spiroplasma* in some species of Staphylinidae, Coccinellidae and Curculionidae (Majerus et al., 1998; Hurst et al., 1999; Tinsley & Majerus, 2006; Weinert et al., 2007; Toju & Fukatsu 2011; Bili et al., 2016), and *Cardinium* has not been detected so far in any beetle species (Zchori-Fein et al., 2004). The coexistence of different endosymbiotic bacteria and their effects on hosts should also be investigated with NGS technologies, which are able to detect bacteria in numerous hosts (e.g. individuals) at once and estimate prevalence of bacteria in various hosts or different tissues. NGS has already been proven to be a powerful tool for detecting undescribed bacteria (e.g. it allowed the identification of new Alphaproteobacteria in *Brontispa longissimi*; Takano et al., 2017). Different endosymbiotic bacteria could have either similar or contrasting effects on beetle species, populations and individuals and could be the greatest overlooked phenomenon in the evolution and ecology of Coleoptera.

In our opinion, beetles are still an insufficiently examined group of *Wolbachia* hosts, especially considering their systematic and ecological diversity. All issues in studies on *Wolbachia* in Coleoptera are generally the same as in other hosts of these bacteria, or *vice versa*; there is no issue that has been or is being studied on *Wolbachia* infection in other (non-beetle) hosts that could not also be examined in beetle hosts. And the extraordinary diversity of beetles (with respect to their diverse systematics at various taxonomic levels, complex phylogenetic relations and extensive ecological relations with each other and numerous other species) makes this group an excellent target for *Wolbachia* studies. The presented summary about *Wolbachia* infection in beetles shows that despite numerous studies, there are still many issues that need to be investigated. We hope that this systematic review will facilitate various future studies on *Wolbachia* infection among beetles.

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References

Ahmed MZ, Li SJ, Xue X, Yin XJ, Ren SX, Jiggins FM, Greeff JM, Qiu BL. 2015. The Intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. *PLOS Pathogens* **11**:19 pp. DOI 10.1371/journal.ppat.1004672

Aikawa T, Anbutsu H, Nikoh N, Kikuchi T, Shibata F, Fukatsu T. 2009. Longicorn beetle that vectors pinewood nematode carries many *Wolbachia* genes on an autosome. *Proceedings of the Royal Society B: Biological Sciences* **276**:3791–3798. DOI 10.1098/rspb.2009.1022.

Anisimova M, Gascuel O. 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Systematic Biology* **55**: 539–552. DOI 10.1080/10635150600755453.

Arthofer W, Riegler M, Avtzis D, Stauffer C. 2009. Evidence for low-titre infections in insect symbiosis: *Wolbachia* in the bark beetle *Pityogenes chalcographus* (Coleoptera, Scolytinae). Environmental Microbiology **11**: 1923–33. DOI: 10.1111/j.1462-2920.2009.01914.x.

Baldo L, Werren JH. 2007. Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. *Current Microbiology* **55**: 81–87. DOI 10.1007/s00284-007-0055-8.

Baldo L, Hotopp JCD, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MCJ, Tettelin H, Werren JH. 2006: Multilocus sequence typing system for the Endosymbiont *Wolbachia pipientis*. *Applied and Environmental Microbiology* **72**: 7098–7110. DOI 10.1128/AEM.00731-06.

Barr KL, Hearne LB, Briesacher S, Clark TL, Davis GE. 2010. Microbial Symbionts in Insects Influence Down-Regulation of Defense Genes in Maize. *PLoS ONE* **5**: e11339. DOI: 10.1371/journal.pone.0011339.

Berasategui A, Axelsson K, Nordlander G, Schmidt A, Borg-Karlson A-K, Gershenzon J, Terenius O, Kaltenpoth M. 2016. The gut microbiota of the pine weevil is similar across Europe and resembles that of other 649 conifer beetles. *Molecular Ecology* **25:** 4014-4031. DOI: 10.1111/mec.14186.

Bili M, Cortesero AM, Mougel C, Gauthier JP, Ermel G, Simon JC, Outreman Y, Terrat S, Mahéo F, Poinsot D. 2016. Bacterial Community Diversity Harboured by Interacting Species. *PLoS ONE* **11**: e0155392. DOI:10.1371/journal.pone.0155392.

Bouchard P, Grebennikov VV, Smith ABT, Douglas H. 2009. Biodiversity of Coleoptera In: Foottit RG, Adler PH, eds. *Insect biodiversity: science and society*. Oxford: Blackwell Publishing, 265–301.

Campbell BC, Bragg TS, Turner C E. 1992. Phylogeny of symbiotic bacteria of four weevil species (Coleoptera: Curculionidae) based on analysis of 16s ribosomal DNA. *Insect Biochemistry and Molecular Biology* **22**:415–421. DOI 10.1016/0965-1748(92)90136-3.

Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C, Genchi C. 2001. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology* **122**:93–103. DOI:10.1017/S0031182000007149.

Chaisiri K, McGarry JW, Morand S, Makepeace B. 2015. Symbiosis in an overlooked microcosm: a systematic review of the bacterial flora of mites. *Parasitology FirstView*, 1–11.

Caspi-Fluger A, Inbar M, Mozes-Daube N, Katzir N, Portnoy V, Belausov E, Hunter MS, Zchori-Fein E. 2012. Horizontal transmission of the insect symbiont *Rickettsia* is plant-mediated. *Proceedings of the Royal Society B: Biological Sciences* **279**:1791–1796. DOI 10.1098/rspb.2011.2095.

Chafee ME, Funk DJ, Harrison RG, Bordenstein SR. 2010. Lateral phage transfer in obligate intracellular bacteria (*Wolbachia*): Verification from natural populations. *Molecular Biology and Evolution* **27**: 501—505. DOI: 10.1093/molbev/msp275.

Chen SJ, Lu F, Cheng JA, Jiang MX, Way MO. 2012. Identification and Biological Role of the Endosymbionts *Wolbachia* in Rice Water Weevil (Coleoptera: Curculionidae). *Environmental Entomology* **41**:469–477. DOI 10.1603/EN11195.

Clark ME, Bailey-Jourdain C, Ferree PM, England SJ, Sullivan W, Windsor DM, Werren JH. 2008. Wolbachia modification of sperm does not always require residence within developing sperm. *Heredity* **101**: 420–428. DOI: 10.1038/hdy.2008.71.

Clark TL, Meinke LJ, Skoda SR, Foster JE. 2001. Occurrence of *Wolbachia* in Selected Diabroticite (Coleoptera: Chrysomelidae) Beetles. *Annals of the Entomological Society of America* **94**:877–885.

Cordaux R, Bouchon D, Greve P. 2011. The impact of endosymbionts on the evolution of host sex-determination mechanisms. *Trends in Genetics* **27**:332–341. DOI 10.1016/j.tig.2011.05.002

Czarnetzki AB, Tebbe CC. 2004. Detection and phylogenetic analysis of *Wolbachia* in Collembola. *Environmental Microbiology* **6**:35–44. DOI 10.1046/j.1462-2920.2003.00537.x.

DeLay B, Mamidala P, Wijeratne A, Wijeratne S, Mittapalli O, Wang J, Lamp W. 2012. Transcriptome analysis of the salivary glands of potato leafhopper, *Empoasca fabae*. *Journal of Insect Physiology* **58**:1626–1634. DOI 10.1016/j.jinsphys.2012.10.002.

Dudek K, Humińska K, Wojciechowicz J, Tryjanowski P. 2017. Metagenomic survey of bacteria associated with the invasive ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae). *European Journal of Entomology* **114**:312–316. DOI 10.14411/eje.2017.038.

Dunning Hotopp J C, Clark ME, Oliveira DCSG, Foster JM, Fischer P, Muñoz Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH. 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* (New York, N.Y.), **317**: 1753–1756. DOI:10.1126/science.1142490

Duron O, Bouchon D, Boutin S, Bellamy L, Zhou LQ, Engelstadter J, Hurst GD. 2008. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biology* **6**:27. DOI 10.1186/1741-7007-6-27.

Elnagdy S, Majerus MEN, Gardener M, Lawson-Handley L. 2013. The direct effects of male-killer infection on fitness of ladybird hosts (Coleoptera: Coccinellidae). *Journal of Evolutionary Biology* **26**:1816–1825. DOI 10.1111/jeb.12186.

Farrell BD. 1998. "Inordinate fondness" explained: why are there so many beetles? *Science* **281**:555–559. DOI 10.1126/science.281.5376.555.

Fialho RF, Stevens L. 2000. Male-killing Wolbachia in a flour beetle. *Proceedings of the Royal Society B: Biological Sciences* **267**:1469–1474. DOI 10.1098/rspb.2000.1166.

Fialho RF, Stevens L. 1996. *Wolbachia* infections in the flour beetle *Tribolium confusum*: evidence for a common incompatibility type across strains. *Journal of Invertebrate Pathology* **67**:195–197. DOI 10.1006/jipa.1996.0032,

Fialho RF, Stevens L. 1997. Molecular evidence for single *Wolbachia* infections among geographic strains of the flour beetle *Tribolium confusum*. *Proceedings of the Royal Society B: Biological Sciences* **264**:1065–1068. DOI 10.1098/rspb.1997.0147.

Floate KD, Coghlin PC, Dosdall L. 2011. A Test using *Wolbachia* Bacteria to Identify Eurasian Source Populations of Cabbage Seedpod Weevil, *Ceutorhynchus obstrictus* (Marsham), in North America. *Environmental Entomology* **40**:818–823. DOI 10.1603/EN10315

Frank JH, Erwin TL & Hemenway RC. 2009. Economically Beneficial Ground Beetles. The specialized predators *Pheropsophus aequinoctialis* (L.) and *Stenaptinus jessoensis* (Morawitz): Their laboratory behavior and descriptions of immature stages (Coleoptera, Carabidae, Brachininae). *Zookeys* **14**: 1-36. DOI: 10.3897/zookeys.14.188.

Funkhouser-Jones LJ, Sehnert SR, Martínez-Rodríguez P, Toribio-Fernández R, Pita M, Bella JL, Bordenstein SR. 2015. *Wolbachia* co-infection in a hybrid zone: discovery of horizontal gene transfers from two *Wolbachia* supergroups into an animal genome. *PeerJ* **3**: e1479. DOI:10.7717/peerj.1479

García-Vázquez D, Ribera I. 2016. The origin of widespread species in a poor dispersing lineage (diving beetle genus Deronectes). *PeerJ* **4**: e2514. DOI: 10.7717/peerj.2514.

Giordano R, Jackson JJ, Robertson HM. 1997. The role of *Wolbachia* bacteria in reproductive incompatibilities and hybrid zones of *Diabrotica* beetles and *Gryllus* crickets. *Proceedings of the National Academy of Sciences of the USA* **94**:11439–11444.

Goodacre SL, Fricke C, Martin OY. 2015. A screen for bacterial endosymbionts in the model organisms *Tribolium castaneum, T.confusum, Callosobruchus maculatus*, and related species. *Insect Science* **22**: 165-177. DOI: 10.1111/1744-7917.12096.

Goodacre SL, Martin OY, Thomas CFG, Hewitt GM. 2006. *Wolbachia* and other endosymbiont infections in spiders. *Molecular Ecology* **15**:517–527. DOI 10.1111/j.1365-294X.2005.02802.x.

Goryachevaa II, Blekhmanb AV, Andrianova BV, Gorelovaa TV, Zakharova IA. 2015. Genotypic Diversity of *Wolbachia* pipientis in Native and Invasive *Harmonia axyridis* Pall., 1773 (Coleoptera, Coccinellidae) Populations. *Russian Journal of Genetics* **8**:731–736.

Goto S, Anbutsu H, Fukatsu T. 2006. Asymmetrical interactions between *Wolbachia* and Spiroplasma endosymbionts coexisting in the same insect host. *Applied and Environmental Microbiology* **72**:4805–4810. DOI 10.1128/AEM.00416-06.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**:307–321. DOI 10.1093/sysbio/syq010.

Hartelt K, Oehme R, Frank H, Brockmann SO, Hassler D, Kimmig P. 2004. Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (Ehrlichia sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in Southern Germany. *International Journal of Medical Microbiology Supplements* **293**:86–92. DOI 10.1016/S1433-1128(04)80013-5.

Heddi A, Grenier AM, Khatchadourian Ch, Charles H, Nardon P. 1999. Four intracellular genomes direct weevil biology: Nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. *Proceedings of the National Academy of Sciences of the USA* **96**:6814–6819. DOI 10.1073/pnas.96.12.6814.

Heintzman PD, Elias SA, Moore K, Paszkiewicz K, Barnes I. 2014. Characterizing DNA preservation in degraded specimens of *Amara alpina* (Carabidae: Coleoptera). *Molecular Ecology Resources* **14**:606–615. DOI 10.1111/1755-0998.12205.

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. 2008. How many species are infected with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiology Letters* **281**:215–220. DOI 10.1111/j.1574-6968.2008.01110.x.

Huigens ME, Almeida RP de, Boons P a H, Luck RF, Stouthamer R. 2004. Natural interspecific and intraspecific horizontal transfer of parthenogenesis–inducing *Wolbachia* in *Trichogramma* wasps. *Proceedings of the Royal Society B: Biological Sciences* **271**:509–515. DOI 10.1098/rspb.2003.2640

Hurst GDD, Bandi C, Sacchi L, Cochrane A, Bertrand D, Karaca I, Majerus MEN.1999. *Adonia variegata* (Coleoptera: Coccinellidae) bears maternally inherited Flavobacteria that kill males only. *Parasitology* **118**:125–134.

Hurst GDD, Jiggins FM. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies : the effects of inherited symbionts. *Proceedings of the National Academy of Sciences USA* **272**:1525–1534. DOI 10.1098/rspb.2005.3056

Hurst GDD, Jiggins FM, von der Schulenburg JHG, Bertrand D, West SA, Goriacheva II, Zakharov IA, Werren JH, Stouthamer R, Majerus MEN. 1999b. Male-killing *Wolbachia* in two species of insect. *Proceedings of the Royal Society B: Biological Sciences* **266**: 735–740. DOI 10.1098/rspb.1999.0698.

Iwase S, Tani S, Saeki Y, Tuda M, Haran J, Skuhrovec J, Takagi M. 2015. Dynamics of infection with *Wolbachia* in Hyperapostica (Coleoptera: Curculionidae) during invasion and establishment. *Biological Invasions* **17**: 3639–3648. DOI: 10.1007/s10530-015-0985-1.

Jäckel R, Mora D, Dobler S. 2013. Evidence for selective sweeps by *Wolbachia* infections: Phylogeny of *Altica* leaf beetles and their reproductive parasites. *Molecular Ecology* **22**: 4241–4255. DOI 10.1111/mec.12389.

Jeong G, Kang T, Park H, Choi J, Hwang S, Kim W, Choi Y, Lee K, Park I, Sim H 2009. *Wolbachia* infection in the Korean endemic firefly, *Luciola unmunsana* (Coleoptera: Lampyridae). *Journal of Asia-Pacific Entomology* **12**:33–36. DOI 10.1016/j.aspen.2008.11.001.

Jeyaprakash A, Hoy MA 2000. Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of 63 arthropod species. *Insect Molecular Biology* **9**: 393–405. DOI 10.1046/j.1365-2583.2000.00203.x.

Kageyama D, Narita S, Imamura T, Miyanoshita A. 2010. Detection and identification of *Wolbachia* endosymbionts from laboratory stocks of stored-product insect pests and their parasitoids. *Journal of Stored Products Research* **46**: 13–19. DOI: 10.1016/j.jspr.2009.07.003.

Kajtoch Ł, Montagna M, Wanat M. 2017. Species delimitation within the Bothryorrhynchapion weevils: multiple evidence from genetics, morphology and ecological associations. *Molecular Phylogenetics and Evolution*. DOI: 10.1016/j.ympev.2017.12.022.

Kajtoch Ł, Korotyaev B, Lachowska-Cierlik D. 2012. Genetic distinctness of parthenogenetic forms of European *Polydrusus* weevils of the subgenus *Scythodrusus*. *Insect Science* **19**:183–194. DOI 10.1111/j.1744-7917.2011.01448.x.

Kawasaki Y, Schuler H, Stauffer C, Lakatos F, Kajimura H. 2016. *Wolbachia* endosymbionts in haplodiploid and diploid scolytine beetles (Coleoptera: Curculionidae: Scolytinae). *Environmental Microbiology Reports* **8**:680–688. DOI 10.1111/1758-2229.12425.

Kawasaki Y, Ito M, Miura K, Kajimura H. 2010. Superinfection of five *Wolbachia* in the alnus ambrosia beetle, *Xylosandrus germanus* (Blandford) (Coleoptera: Curuculionidae). *Bullettin of Entomological Research* **100**:231–239. DOI 10.1017/S000748530999023X.

Keller GP, Windsor DM, Scucedo JM, Werren JH. 2004. Reproductive effects and geographical distributions of two *Wolbachia* strains infecting the Neotropical beetle, *Chelymorpha alternans* Boh. (Chrysomelidae, Cassidinae). *Molecular Ecology* **13**:2405–2420. DOI 10.1111/j.1365-294X.2004.02213.x.

Kikuchi Y, Fukatsu T. 2003. Diversity of *Wolbachia* endosymbionts in heteropteran bugs. *Applied and Environmental Microbiology* **69**:6082-6090. DOI 10.1128/AEM.69.10.6082-6090.2003

Kolasa M, Montagna M, Mereghetti V, Kubisz D, Mazur MA, Kajtoch Ł. 2017. Preliminary evidence of *Wolbachia* horizontal transmission between *Crioceris* leaf beetles and *Asparagus* host plants. *European Journal of Entomology* **114**:446–454. DOI 10.14411/eje.2017.057

Kondo N, Ijichi N, Shimada M, Fukatsu T. 2002. Prevailing triple infection with *Wolbachia* in *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Molecular Ecology* **11**:167–180. DOI 10.1046/j.0962-1083.2001.01432.x

Kondo N, Shimada M, Fukatsu T. 1999. High prevalence of *Wolbachia* in the azuki bean beetle *Callosobruchus chinensis* (Coleoptera, Bruchidae). *Zoological Science* **16**:955–962. DOI 10.2108/zsj.16.955

Kondo N, Shimada M, Fukatsu T. 2005. Infection density of *Wolbachia* endosymbiont affected by co-infection and host genotype. *Biology Letters* **1**:488–491. DOI 10.1098/rsbl.2005.0340

Kondo N, Tuda M, Toquenaga Y, Lan YC, Buranapanichpan S, Horng SB, Shimada M, Fukatsu T. 2011. *Wolbachia* infections in world populations of bean beetles (Coleoptera: Chrysomelidae: Bruchinae) infesting cultivated and wild legumes. *Zoological Science* **28**:501–508. DOI 10.2108/zsj.28.501

Koutsovoulos G, Makepeace B, Tanya VN, Blaxter M. 2014. Palaeosymbiosis Revealed by Genomic Fossils of Wolbachia in a Strongyloidean Nematode. *PLOS Genetics*: **10**(6), e1004397. doi:10.1371/journal.pgen.1004397

Kubisz D, Kajtoch Ł, Mazur MA, Lis A, Holecová M. 2012. Conservation genetics of highly isolated populations of xerothermic *Crioceris quatuordecimpunctata* (Coleoptera: Chrysomelidae). *Invertebrate Biology* **131**:333–344. DOI 10.1111/j.1744-7410.2012.00276.x.

Lachowska-Cierlik D, Kajtoch Ł, Knutelski S. 2010. Occurrence of *Wolbachia* in central European weevils: correlations with host systematics, ecology and biology. *Entomologia Experimentalis et Applicata* **14**:105–118. DOI 10.1111/j.1570-7458.2010.00974.x.

Lawson ET, Mousseau TA, Klaper R, Hunter MD, Werren JH. 2001. *Rickettsia* associated with male-killing in a buprestid beetle. *Heredity* **86**:497–505. DOI 10.1046/j.1365-2540.2001.00848.x.

Li YY, Fields PG, Pang B, Coghlin PC, Floate KD. 2015. Prevalence and diversity of Wolbachia bacteria infecting insect pests of stored products. *Journal of Stored Products Research* **62**: 93-100. DOI: 10.1016/j.jspr.2009.07.003.

Li YY, Fields PG, Pang BP, Floate KD. 2016. Effects of tetracycline and rifampicin treatmens on the fecundity of the *Wolbachia*-infected host, *Tribolium confusum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology* **109**:1458–1464. DOI 10.1093/jee/tow067.

Li SJ, Ahmed MZ, Lv N, Shi PQ, Wang XM, Huang JL, Qiu BL. 2016. Plant–mediated horizontal transmission of *Wolbachia* between whiteflies. *The ISME Journal* **11**:1019–1028. DOI 10.1038/ismej.2016.164

Lindsey AR, Bordenstein SR, Newton IL, Rasgon JL. 2016. Wolbachia pipientis should not be split into multiple species: A response to Ramírez-Puebla et al.,, "Species in *Wolbachia*? Proposal for the designation of '*Candidatus* Wolbachia bourtzisii', '*Candidatus* Wolbachia onchocercicola', '*Candidatus* Wolbachia blaxteri', '*Candidatus* Wolbachia brugii', '*Candidatus* Wolbachia taylori' '*Candidatus* Wolbachia collembolicola' and '*Candidatus* Wolbachia multihospitum' for the different species within *Wolbachia* supergroups". *Systematic and Applied Microbiology* **39**:220–222. DOI 10.1016/j.syapm.2016.03.001.

Lu F, Kang XY, Lorenz G, Espino L, Jiang MX, Way MO 2014. Culture-independent analysis of bacterial communities in the gut of rice water weevil (Coleoptera: Curculionidae). *Annals of the Entomological Society of America* **10**,592–600. DOI 10.1603/AN13145.

Majerus TMO, Majerus MEN, Knowles B, Wheeler J, Bertrand D, Kuznetsov VN, Ueno H, Hurst GDD. 1998. Extreme variation in the prevalence of inherited male-killing microorganisms between three populations of *Harmonia axyridis* (Coleoptera: Coccinellidae). *Heredity* **81**:683–691. DOI 10.1046/j.1365-2540.1998.00438.x.

Majerus MEN, Majerus TMO. 2000. Multiple causes of male-killing in a single sample of the 2-spot ladybird, *Adalia bipunctata* (Coleoptera:Coccinellidae) from Moscow. *Heredity* **84**, 605–609. DOI 10.1046/j.1365-2540.2000.00710.x

Malloch G, Fenton B, Butcher RD. 2000. Molecular evidence for multiple infections of a new subgroup of *Wolbachia* in the European raspberry beetle *Byturus tomentosus*. *Molecular Ecology* **9**:77–90.

Mariño Y, Verle Rodrigues JC, Bayman P. 2017. *Wolbachia* Affects Reproduction and Population Dynamics of the Coffee Berry Borer (*Hypothenemus hampei*): Implications for *Biological Control Insects* **8**:8. DOI 10.3390/insects8010008.

Mazur M.A., Holecová M., Lachowska-Cierlik D., Lis A., Kubisz D., Kajtoch Ł. 2016. Selective sweep of *Wolbachia* and parthenogenetic host genomes on the example of the weevil *Eusomus ovulum*. *Insect Molecular Biology* **25**:701–711. DOI 10.1111/imb.1225.

Mazur MA, Kubisz D, Kajtoch Ł. 2014. Restricted geographic distribution and low genetic distinctiveness of steppic *Crioceris quinquepunctata* (Coleoptera: Chrysomelidae) populations in central-east Europe. *Entomologica Fennica* **25**:103–111.

Ming QL, Shen JF, Cheng C., Liu CM, Feng ZJ. 2015. *Wolbachia* infection dynamics in *Tribolium confusum* (Coleoptera: Tenebrionidae) and their effects on host mating behavior and reproduction. *Journal of Economic Entomology* **108**:1408–1415. DOI 10.1093/jee/tov053.

Minh BQ, Nguyen NAT, von Haeseler A. 2013. Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution* **30**:1188–1195. DOI 10.1093/molbev/mst024.

Moher D, Liberati A, Tetzlaff J, Altman DG. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine* **151**:264–269 DOI 10.7326/0003-4819-151-4-200908180-00135.

Montagna M, Chouaia B, Sacchi L, Porretta D, Martin E, Giorgi A, Lozzia GC, Epis SA. 2014. New Strain of *Wolbachia* in an Alpine Population of the Viviparous *Oreina cacaliae* (Coleoptera: Chrysomelidae). *Environmental Entomology* **43**:913–922. DOI 10.1603/EN13228.

Nikoh N, Tanaka K, Shibata F, Kondo N, Hizume M, Shimada M, Fukatsu T. 2008. *Wolbachia* genome integrated in an insect chromosome: evolution and fate of laterally transferred endosymbiont genes. *Genome Research* **18**:272–280. DOI 10.1101/gr.7144908.

Numajiri Y, Kondo NI, Toquenaga Y. 2017. Melanic mutation causes a fitness decline in bean beetles infected by Wolbachia. *Entomologia Experimentalis et Applicata* **164**: 54-65. DOI: 10.1111/eea.12588.

O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibity in insects. *Proceedings of National Academy of Sciences of the USA* **89**:2699–2702.

Okayama K, Katsuki M, Sumida Y, Okada K. 2016. Costs and benefits of symbiosis between a bean beetle and *Wolbachia*. *Animal Behaviour* **119**:19–26. DOI 10.1016/j.anbehav.2016.07.004.

O'Neill SL. 2007. *Wolbachia*–host interactions: connecting phenotype to genotype. *Current Opinion in Microbiology* **10**:221–224. DOI 10.1016/j.mib.2007.05.002.

Pankewitz F, Zöllmer A, Hilker M, Gräser Y. 2007. Presence of *Wolbachia* in Insect Eggs Containing Antimicrobially Active Anthraquinones. *Microbial Ecology* **54**: 713–721. DOI: 10.1007/s00248-007-9230-5.

Perotti MA, Young DK, Braig HR. 2016. The ghost sex-life of the paedogenetic beetle *Micromalthus debilis*. *Scientific reports* **6:** 27364. DOI: 10.1038/srep27364.

Piper RW, Compton SG, Rasplus JY, Piry S. 2001. The species status of *Cathormiocerus britannicus*, an endemic, endangered British weevil. *Biological Conservation* **101**:9–13. DOI 10.1016/S0006-3207(01)00048-9

Prakash BM & Puttaraju HP. 2006. *Wolbachia* endosymbiont in some insect pests of sericulture. *Current Science* **90:** 1671-1674.

Ramirez-Puebla ST, Servin-Garciduenas LE, Ormeno-Orrillo E, Vera-Ponce de Leon A, Rosenblueth M, Delaye L, Martinez J, Martinez-Romero E. 2015. Species in *Wolbachia*? Proposal for the designation of '*Candidatus* Wolbachia bourtzisii', '*Candidatus* Wolbachia onchocercicola', '*Candidatus* Wolbachia blaxteri', '*Candidatus* Wolbachia brugii', '*Candidatus* Wolbachia taylori' '*Candidatus* Wolbachia collembolicola' and '*Candidatus* Wolbachia multihospitum' for the different species within *Wolbachia* supergroups. *Systematic and Applied Microbiology* **38**:390–399. DOI 10.1016/j.syapm.2015.05.005.

Rodriguero MS, Confalonieri VA, Guedes JVC, Lanteri AA. **2010a.** *Wolbachia* infection in the tribe Naupactini (Coleoptera, Curculionidae): association between thelytokous parthenogenesis and infection status. *Insect Molecular Biology* **19**:631–640. DOI 10.1111/j.1365-2583.2010.01018.x.

Rodriguero MS, Lanteri AA, Confalonieri VA. 2010b. Mito-nuclear genetic comparison in a *Wolbachia* infected weevil: insights on reproductive mode, infection age and evolutionary forces shaping genetic variation. *BMC Evolutionary Biology* **10**:340. DOI 10.1186/1471-2148-10-340.

Roehrdanz R, Levine E 2007. *Wolbachia* bacterial infections linked to mitochondrial DNA reproductive isolation among populations of northern corn rootworm (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America* **100**:522–531. DOI 10.1603/0013-8746(2007)100[522:WBILTM]2.0.CO;2.

Roehrdanz R, Olson D, Bourchier R, Sears S, Cortilet A, Fauske G 2006. Mitochondrial DNA diversity and *Wolbachia* infection in the flea beetle *Aphthona nigriscutis* (Coleoptera: Chrysomelidae): an introduced biocontrol agent for leafy spurge. *Biological Control* **37**:1–8. DOI 10.1016/j.biocontrol.2005.12.004.

Roehrdanz RL, Wichmann SGS. 2013. *Wolbachia* wsp gene clones detect the distribution of *Wolbachia* variants and wsp hypervariable regions among 160 individuals of a multistrain infected population of *Diabrotica barberi* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America* **106**:329–338. DOI 10.1603/AN12118.

Roehrdanz R, Olson D, Bourchier R, Sears S, Cortilet A, Fauske G. 2006. Mitochondrial DNA diversity and *Wolbachia* infection in the flea beetle *Aphthona nigriscutis* (Coleoptera : Chrysomelidae): An introduced biocontrol agent for leafy spurge. *Biological Control* **37**:1–8. DOI 10.1016/j.biocontrol.2005.12.004.

Russell JA. 2012. The ants (Hymenoptera: Formicidae) are unique and enigmatic hosts of prevalent *Wolbachi*a (Alphaproteobacteria) symbionts. *Myrmecological News* **16**: 7–23.

Sharaf K, Hadid Y, Pavliček T, Nevo E. 2013. Local genetic population divergence in a saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera, Cucujidae). *Journal of Stored Products Research* **53**:72–76.

Sharaf K, Horovă L, Pavliček T, Nevo E, Bureš P. 2010. Genome size and base composition in *Oryzaephilius surinamensis* (Coleoptera: Sylvanidae) (Cikeiotera: Sylvanidae) and differences between native (feral) and silo pest populations in Israel. *Journal of Stored Products Research* **46:**34–37.

Shoemaker DD, Machado CA, Molbo D, Werren JH, Windsor DM, Herre EA. 2002. The distribution of *Wolbachia* in fig wasps: correlations with host phylogeny, ecology and population structure. *Proceedings of the Royal Society of London B: Biological Sciences* **269**:2257–2267. DOI 10.1098/rspb.2002.2100.

Smith MA, Bertrand C, Crosby K, Eveleigh ES, Fernandez-Triana J, Fisher BL, Gibbs J, Hajibabaei M, Hallwachs W, Hind K, Hrcek J, Huang DW, Janda M, Janzen DH, Li Y, Miller SE, Packer L, Quicke D, Ratnasingham S, Rodriguez J, Rougerie R, Shaw MR, Sheffield C, Stahlhut JK, Steinke D, Whitfield J, Wood M, Zhou X. 2012. Wolbachia and DNA Barcoding Insects: Patterns, Potential, and Problems. *PLoS ONE* **7**:e36514. DOI 10.1371/journal.pone.0036514.

Son Y, Luckhart S, Zhang X, Lieber MJ, Lewis EE. 2008. Effects and implications of antibiotic treatment on *Wolbachia* - infected vine weevil (Coleoptera: Curculionidae). *Agricultural and Forest Entomology* **10**:147–155. DOI 10.1111/j.1461-9563.2008.00369.x.

Sontowski R, Bernhard D, Bleidorn C, Schlegel M, Gerth M. 2015. *Wolbachia* distribution in selected beetle taxa characterized by PCR screens and MLST data. *Ecology and Evolution* **5**:4345–4353. DOI 10.1002/ece3.1641.

Stahlhut JK, Desjardins CA, Clark ME, Baldo L, Russell JA, Werren JH, Jaenike J. 2010. The mushroom habitat as an ecological arena for global exchange of *Wolbachia*. *Molecular Ecology* **19**:1940–1952. DOI 10.1111/j.1365-294X.2010.04572.x.

Stenberg, P., Lundmark, M., 2004. Distribution, mechanisms and evolutionary significance of clonality and polyploidy in weevils. *Agricultural and Forest Entomology* **6**:259–266. DOI 10.1111/j.1461-9555.2004.00231.x.

Stouthamer R, Breeuwer JAJ, Hurst GDD 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* **53**:71–102. DOI 10.1146/annurev.micro.53.1.71.

Tagami Y, Miura K. 2004. Distribution and prevalence of *Wolbachia* in Japanese populations of Lepidoptera. *Insect Molecular Biology* **13**:359–364. DOI 10.1111/j.0962-1075.2004.00492.x.

Takano SI, Tuda M, Takasu K, Furuya N, Imamura Y, Kim S, Tashiro K, Iiyama K, Tavares M, Amaral AC. 2017. Unique clade of alphaproteobacterial endosymbionts induces complete cytoplasmic incompatibility in the coconut beetle. Proceedins of National Academy of Sciences USA **114**: 6110–6115. DOI: 10.1073/pnas.1618094114.

Taylor MJ, Hoerauf A. 1999. *Wolbachia* bacteria of filarial nematodes. *Parasitology Today* **15**:437–442. DOI 10.1016/S0169-4758(99)01533-1.

Tinsley MC, Majerus MEN. 2006. A new male-killing parasitism: *Spiroplasma* bacteria infect the ladybird beetle *Anisosticta novemdecimpunctata* (Coleoptera: Coccinellidae). *Parasitology* **132**:757–765. DOI: 10.1017/S0031182005009789.

Toju H, Fukatsu T. 2011. Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: Relevance of local climate and host plants. *Molecular Ecology* **20**: 853–868. DOI: 10.1111/j.1365-294X.2010.04980.x.

Toju H, Tanabe AS, Notsu Y, Sota T, Fukatsu T. 2013. Diversification of endosymbiosis: replacements, co-speciation and promiscuity of bacteriocyte symbionts in weevils. *The ISME Journal* **7**: 1378-1390. DOI: 10.1038/ismej.2013.27.

Toševski I, Caldara R, Jović J, Hernández-Vera G, Baviera C, Gassman A, Emerson BC. 2015. Host associated genetic divergence and taxonomy in the *Rhinusa pilosa* Gyllenhal species complex: an integrative approach. *Systematic Entomology* **40**: 268–287. DOI: 10.1111/syen.12109.

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* **44**:W232–W235. DOI 10.1093/nar/gkw256.

Vega FE, Benavides P, Stuart J, O'Neill SL. 2002. *Wolbachia* infection in the coffee berry borer (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* **95**:374–378. DOI 10.1603/0013-8746(2002)095[0374:WIITCB]2.0.CO;2.

Weeks AR, Reynolds KT, Hoffmann AA 2002. *Wolbachia* dynamics and host effects: What has (and has not) been demonstrated? *Trends of Ecology & Evolution* **17**:257–262. DOI 10.1016/S0169-5347(02)02480-1.

Weinert LA, Tinsley MC, Temperley M, Jiggins FM. 2007. Are we underestimating the diversity and incidence of insect bacterial symbionts? A case study in ladybird beetles. *Biology Letters* **3**: 678–681. DOI: 10.1098/rsbl.2007.0373.

Werren JH, Hurst GDD, Zhang W, Breeuwer JA, Stouthamer R, Majerus ME. 1994. Rickettsial relative associated with male killing in the ladybird beetle (*Adalia bipunctata*). *Journal Bacteriology* **176**:388–394. DOI 10.1128/jb.176.2.388-394.1994.

Werren JH, Windsor D, Guo L 1995. Distribution of *Wolbachia* among neotropical arthropods. *Proceedings of the Royal Society B: Biological Sciences* **262**:197–204. DOI 10.1098/rspb.1995.0196.

Werren, JH, O'Neill, SL. 1997. The evolution of heritable symbionts. In: O'Neill SL, Hoffmann AA, Werren, JH, eds). *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford:Oxford University Press, 1–41.

Werren JH. 1997. Biology of *Wolbachia. Annual Review of Entomology* **42**:587–609. DIO 10.1146/annurev.ento.42.1.587.

White JA, Richards NK, Laugraud A, Saeed A, Curry MM, McNeill MR. 2015. Endosymbiotic candidates for parasitoid defense in exotic and native New Zealand weevils. *Microbial Ecology* **70**: 274–286. DOI: 10.1007/s00248-014-0561-8.

Xue H-J, Li W-Z, Nie R-E, Yang X-K. 2011. Recent Speciation in Three Closely Related Sympatric Specialists: Inferences Using Multi-Locus Sequence, Post-Mating Isolation and Endosymbiont Data. *PLoS ONE* **6**: e27834. DOI:10.1371/journal.pone.0027834.

Yun Y, Peng Y, Liu FX, Lei C. 2011.*Wolbachia* screening in spiders and assessment of horizontal transmission between predator and prey. *Neotropical Entomology* **40**:164–169. DOI 10.1590/S1519-566X2011000200002.

Zchori-Fein E, Borad C, Harari AR. 2006. Oogenesis in the date stone beetle, Coccotrypesdactyliperda, depends on symbiotic bacteria. *Physiological Entomology* **31:** 164–169. DOI: 10.1111/j.1365-3032.2006.00504.x.

Zchori-Fein E, Perlman SJ. 2004. Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology* **13**:2009–2016. DOI 10.1111/j.1365-294X.2004.02203.x.

Zhang X, Luckhart S, Tu J, Pfeiffer DG. 2010. Analysis of *Wolbachia* strains associated with *Conotrachelus nenuphar* (Coleoptera: Curculionidae) in the eastern United States. *Environmental Entomology* **39**:396-405. DOI 10.1603/EN09276.

Zug R, Hammerstein P. 2012. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS One* **7**:e38544. DOI 10.1371/journal.pone.0038544.

Table 1(on next page)

Image of share of *Wolbachia* infected species among families and genera of examined beetles.

Table 1: Share of *Wolbachia* infected species among families and genera of examined beetles. Only taxonomic groups for which at least two species were tested are presented.

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family	N of examined	% of infected	genus	N of examined	% of infected	genus	N of examined	% of infected
Buprestidae	61	23.0	*Archarius*	6	16.7	*Ilybius*	2	0.0
Chrysomelidae	84	45.2	*Atrichonotus*	2	50.0	*Julodis*	2	0.0
Curculionidae	137	41.6	*Barypeithes*	9	11.0	*Koreoculio*	2	50.0
Dytiscidae	36	16.7	*Brachysomus*	4	0.0	*Laccophilus*	2	0.0
Gyrinidae	3	33.3	*Buprestis*	3	0.0	*Limnebius*	7	28.6
Haliplidae	2	50.0	*Byturus*	3	33.0	*Longitarsus*	3	100.0
Hydraenidae	27	63.0	*Callosbruchus*	3	33.3	*Meliboeus*	2	0.0
Hydrophilidae	12	16.7	*Callosobruchus*	7	33.0	*Naupactus*	16	69.0
Noteridae	2	100.0	*Capnodis*	3	33.3	*Neoglanis*	2	0.0
Tenebrionidae	11	9.1	*Charidotella*	2	50.0	*Ochthebius*	12	41.7
subfamily	N of examined	% of infected	*Chrysobothris*	3	33.3	*Oreina*	5	80.0
Bruchinae	24	16.7	*Crioceris*	5	40.0	*Otiorhynchus*	4	50.0
Galerucinae	12	25.0	*Curculio*	23	17.4	*Pantomorus*	3	100.0
Curculionidae	36	16.7	*Cyanapion*	6	50.0	*Polydrosus*	4	75.0
Scolytinae	23	34.8	*Deronectes*	11	45.4	*Rhantus*	2	0.0
genus	N of examined	% of infected	*Diabrotica*	12	25.0	*Rhinusa*	3	33.3
Acalymma	2	100.0	*Dorytomus*	3	67.0	*Sciaphobus*	2	50.0
Acmaeodera	5	0.0	*Eurymetopus*	2	100.0	*Sitophilus*	3	100.0
Acmaeoderella	4	0.0	*Gyrinus*	3	33.0	*Sphenoptera*	11	9.1
Agabus	6	16.7	*Haliplus*	3	33.0	*Strophosoma*	3	67.0
Agrilus	34	17.6	*Helophorus*	3	0.0	*Trachypteris*	2	0.0
Altica	16	88.0	*Hydraena*	24	33.3	*Trachys*	6	16.7
Anthaxia	6	16.7	*Hydroporus*	5	0.0	*Tribolium*	8	12.5
Aramigus	3	100.0	*Hygrotus*	5	20.0			

Figure 1(on next page)

Prisma flow-diagram for literature on Wolbachia-Coleoptera relations included in this study.

Figure 1: Prisma flow-diagram (see Moher et al., 2009) for literature on Wolbachia-Coleoptera relations included in this study.

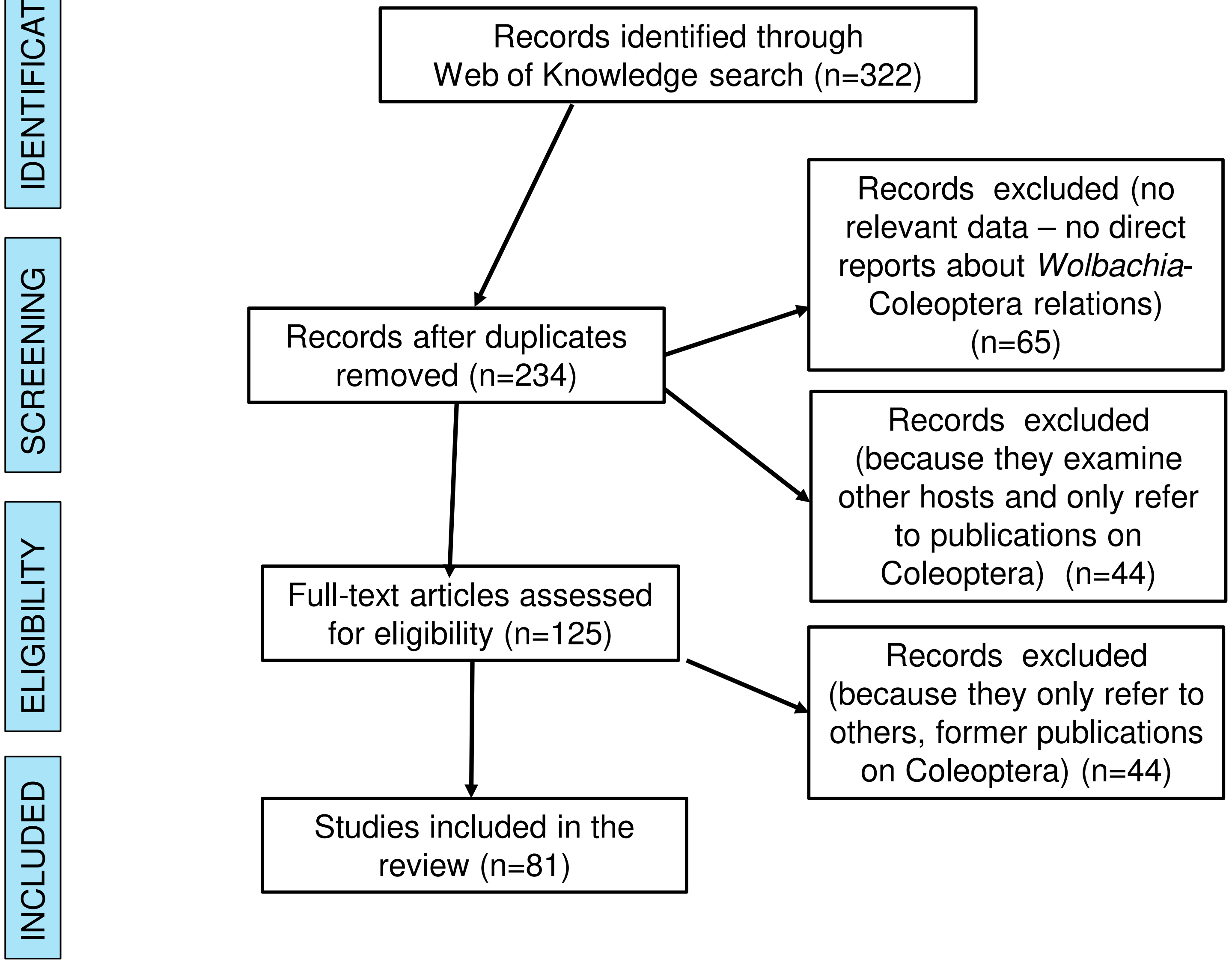

Figure 1 Prisma flow-diagram (see Moher et al., 2009) for literature included in this study. Flowdiagram for literature on *Wolbachia*-Coleoptera relations included in this study.

Figure 2(on next page)

Image of change in the number of publications considering *Wolbachia* infection among Coleoptera.

Figure 2: Change in the number of publications considering *Wolbachia* infection among Coleoptera.

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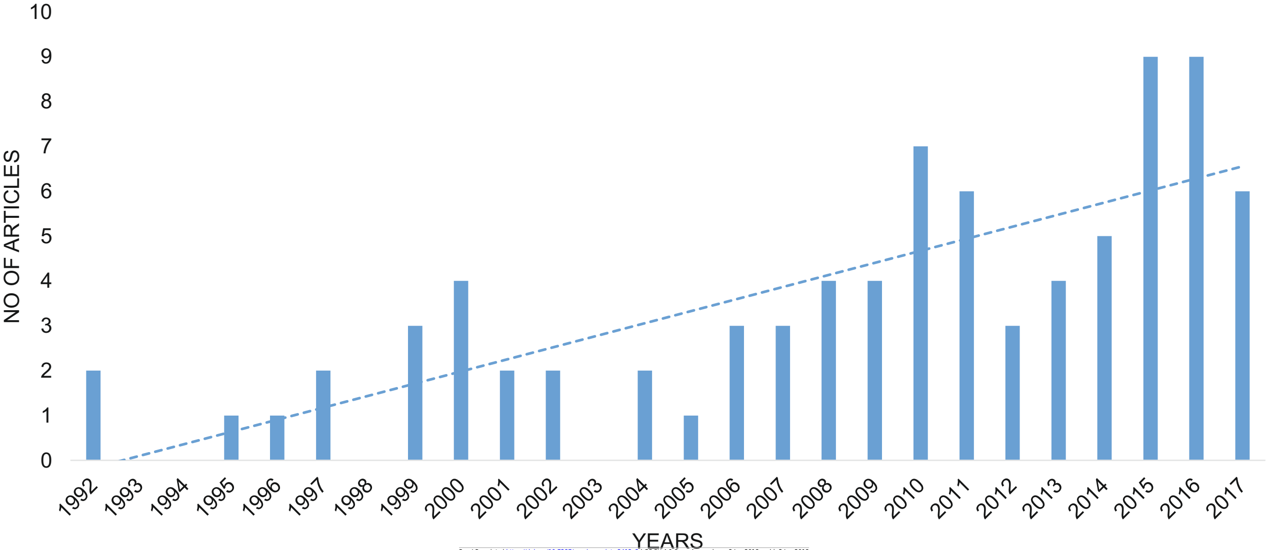

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Figure 3(on next page)

Image of number of publications that described *Wolbachia* infection among Coleoptera and number of infected beetle species.

Figure 3: Number of publications that described *Wolbachia* infection among Coleoptera and number of infected beetle species. Both are shown with respect to the zoogeography of the examined hosts (from which continent the host was collected).)

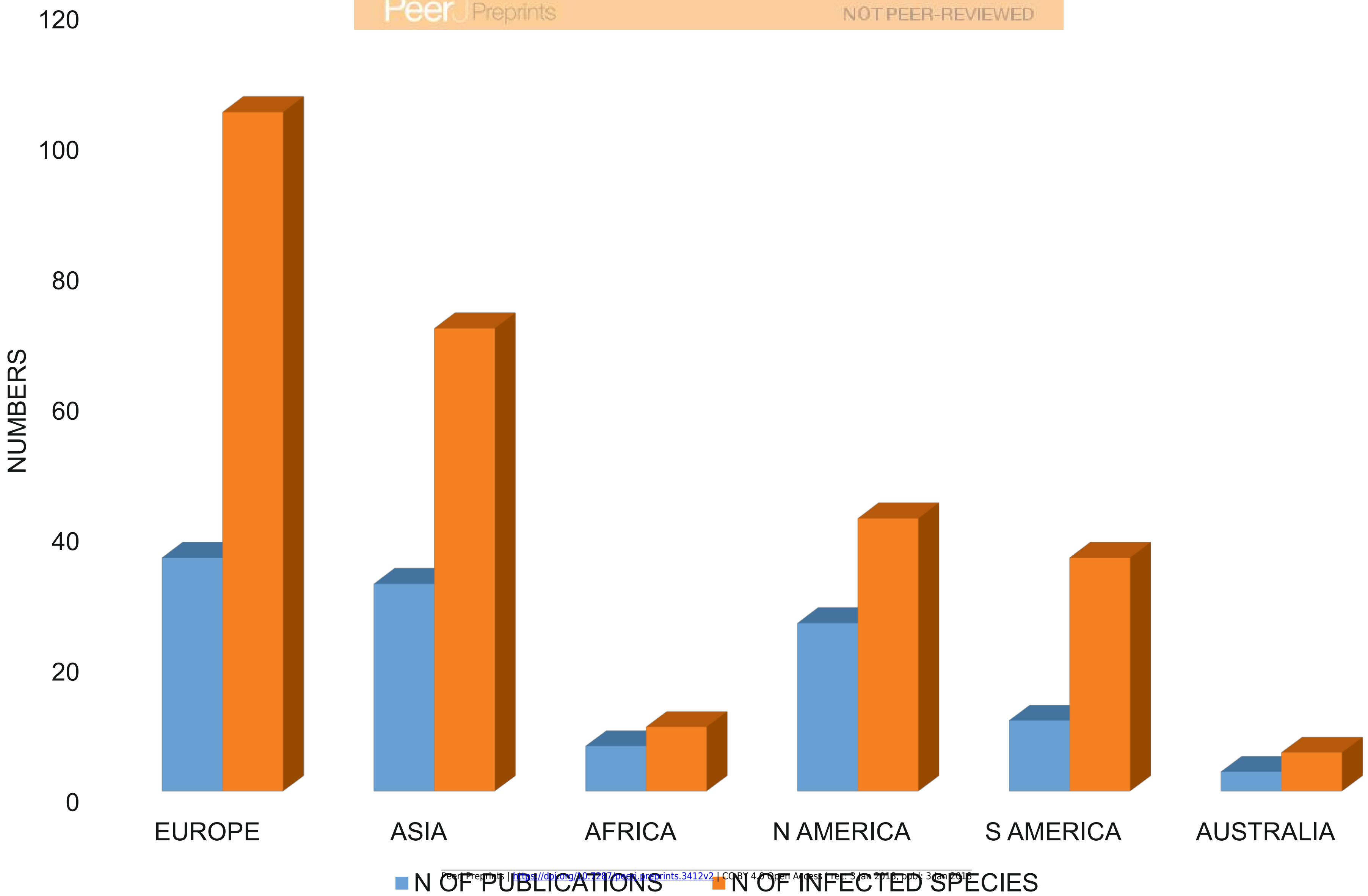
120
100
80
60
40
20
0
NUMBERS
EUROPE
ASIA
AFRICA
N AMERICA
S AMERICA
AUSTRALIA
■ N OF PUBLICATIONS
■ N OF INFECTED SPECIES

Figure 4(on next page)

Image of numbers of sites and numbers of individuals of beetles examined with respect to *Wolbachia* infection.

Figure 4: Numbers of sites and numbers of individuals of beetles examined with respect to *Wolbachia* infection. P – Man-Whitney test p-values.

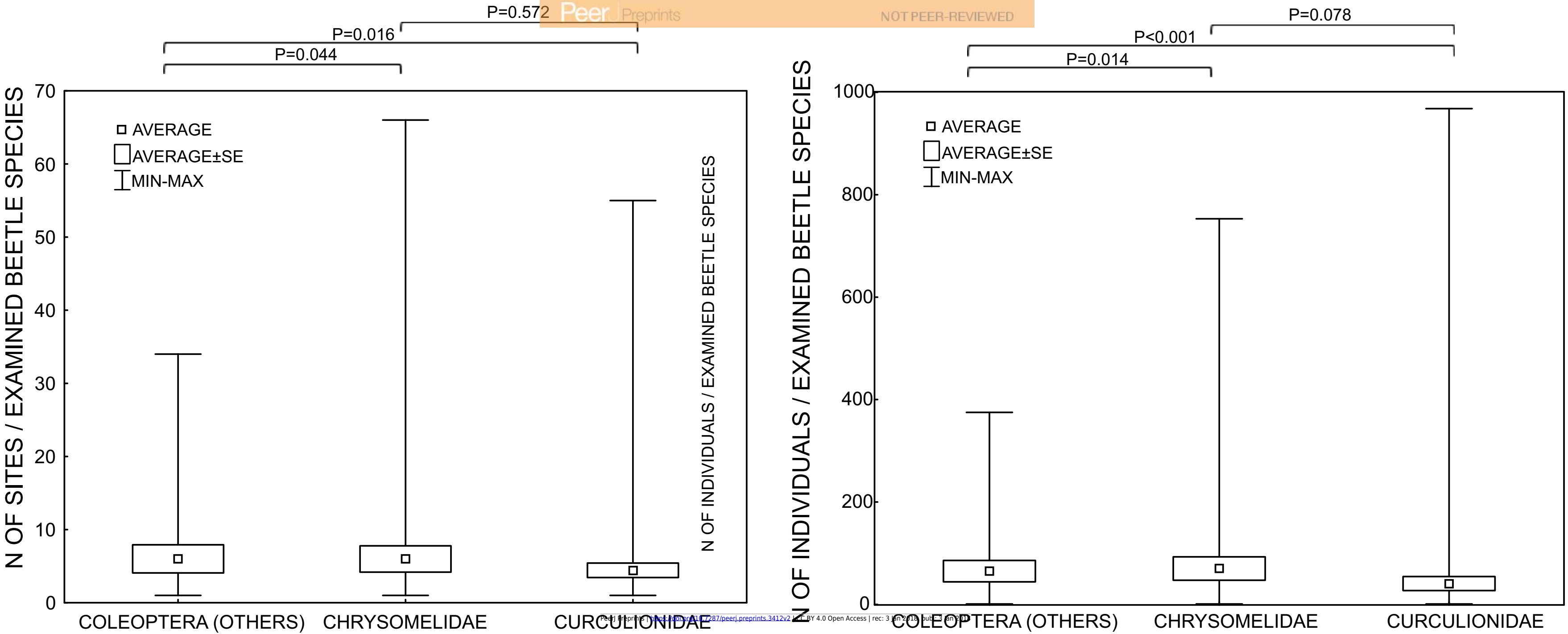

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Figure 5(on next page)

Image of shares of *Wolbachia* genes used in studies on *Wolbachia* infection among Coleoptera.

Figure 5: Shares of *Wolbachia* genes used in studies on *Wolbachia* infection among Coleoptera.

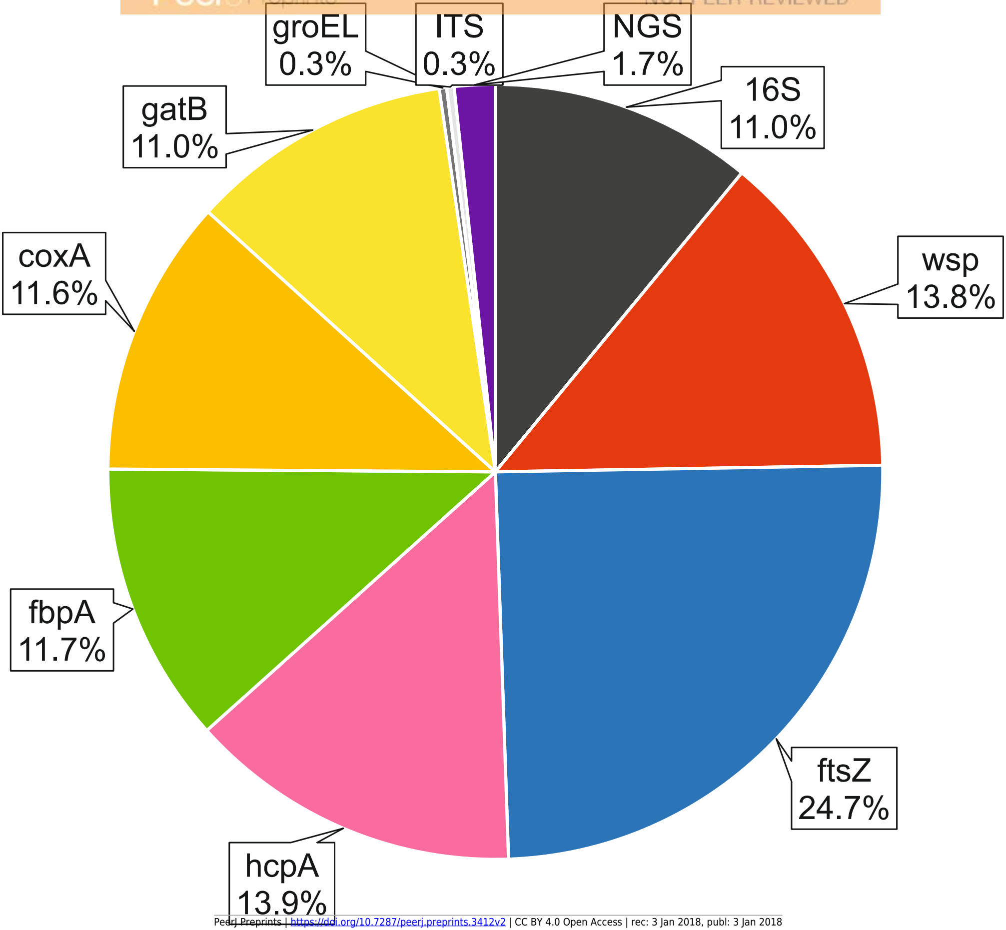

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Figure 6(on next page)

Image of shares of *Wolbachia* infected beetle species across the examined families of Coleoptera.

Figure 6: Shares of *Wolbachia* infected beetle species across the examined families of Coleoptera. The numbers presented after the family names indicate the number of infected species.

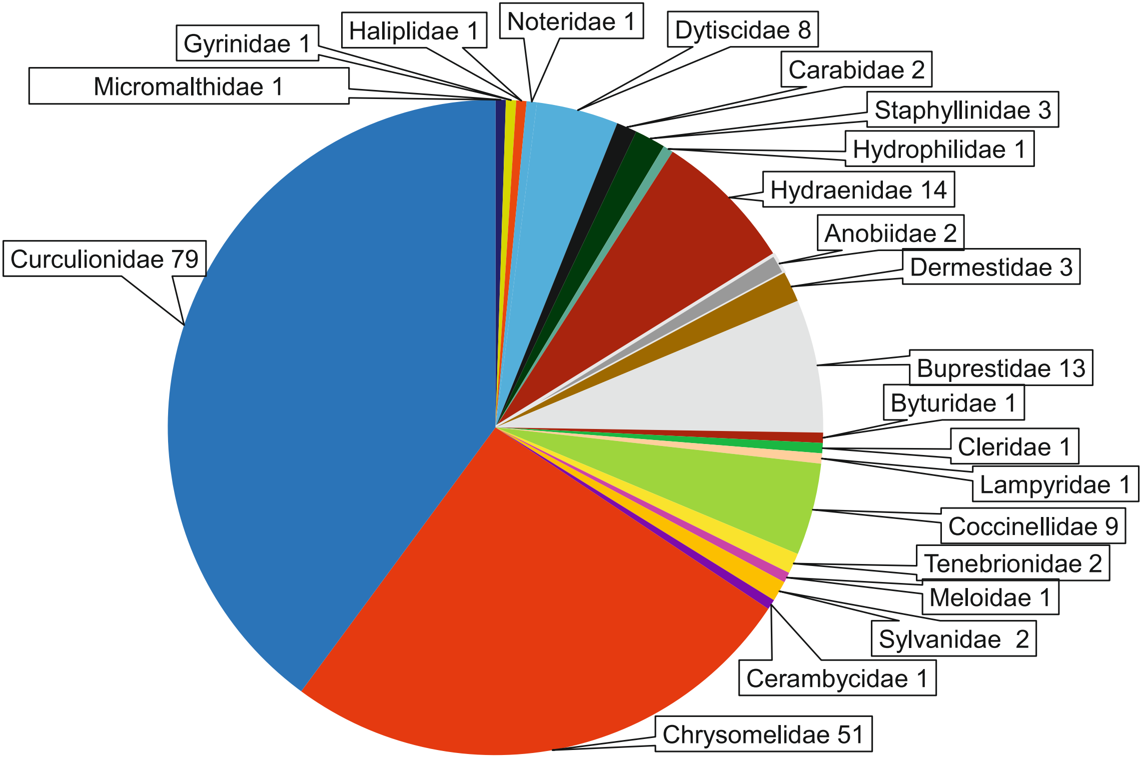

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Figure 7(on next page)

Image of shares of beetles infected by *Wolbachia* supergroups (A, B, F).

Figure 7: Shares of beetles infected by *Wolbachia* supergroups (A, B, F). [Beetle photographs are from ICONOGRAPHIA COLEOPTERORUM POLONIAE (© Copyright by Prof. Lech Borowiec]

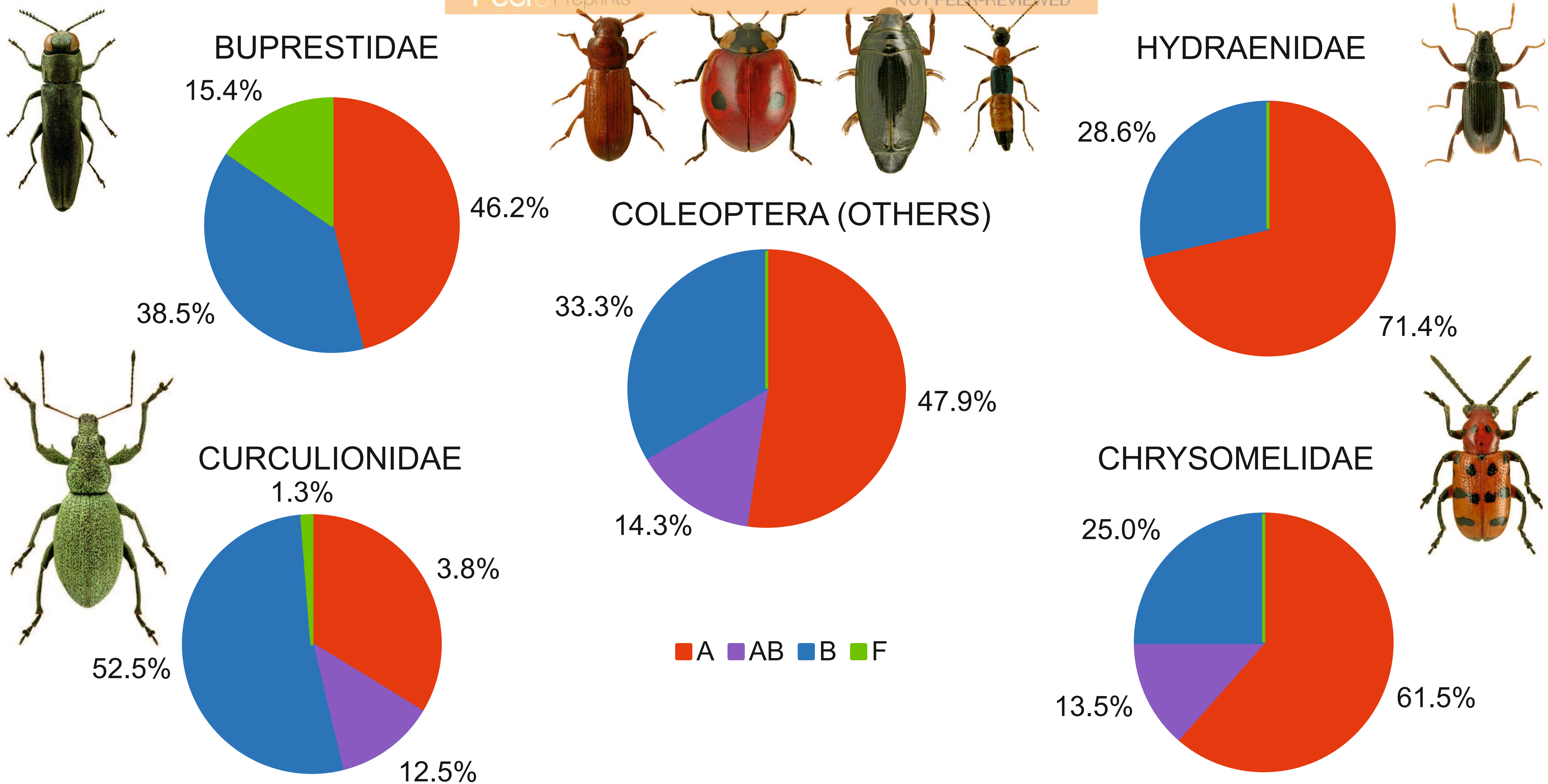

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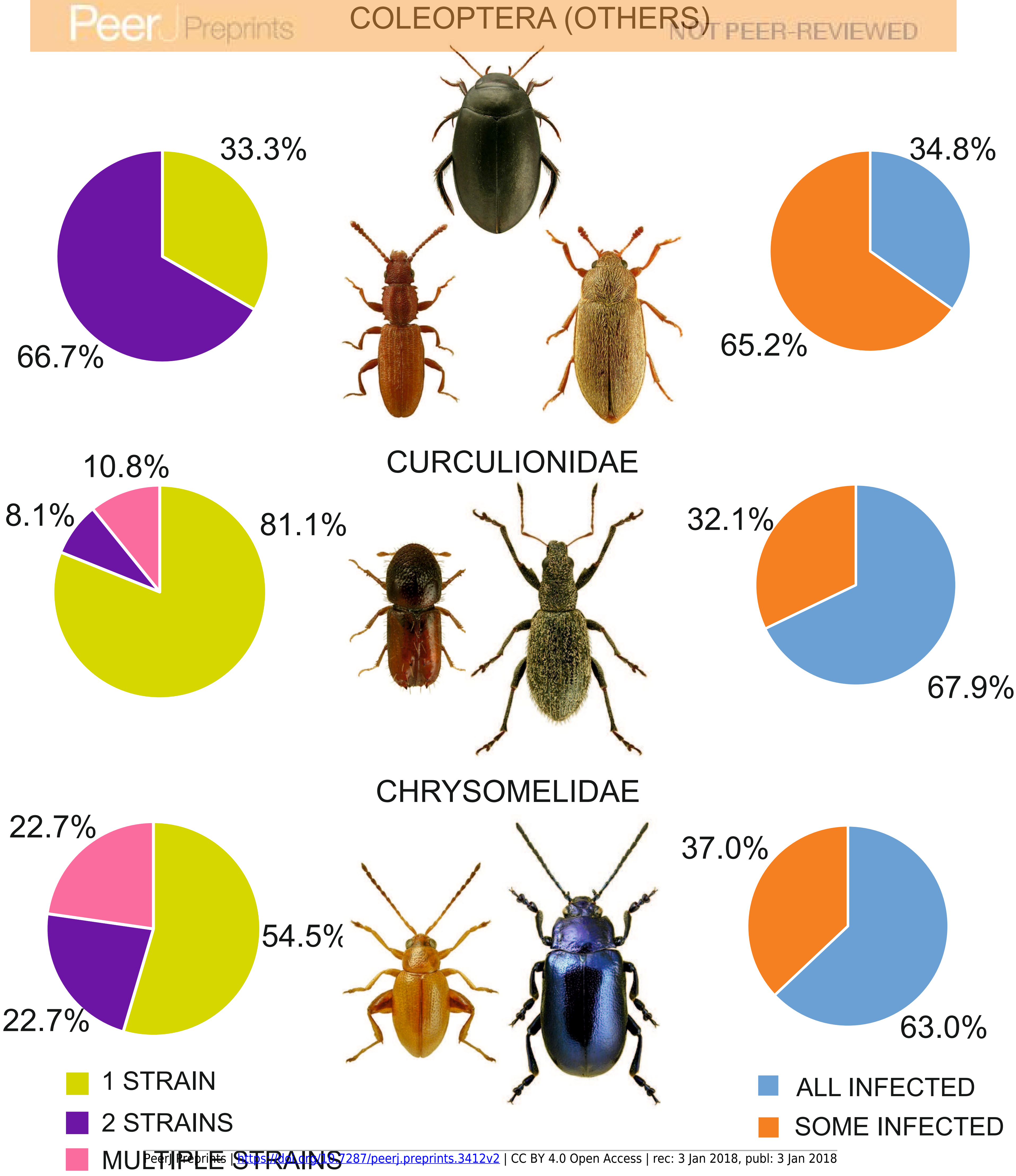
Figure 8(on next page)

Image of diversity of *Wolbachia* infection in Coleoptera with respect to shares of infected individuals within species and numbers of strains found in beetles.

Figure 8: Diversity of *Wolbachia* infection in Coleoptera with respect to shares of infected individuals within species and numbers of strains found in beetles. [Beetle photographs are from ICONOGRAPHIA COLEOPTERORUM POLONIAE (© Copyright by Prof. Lech Borowiec]



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