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## ***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”**

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In their recent paper, Ramírez-Puebla et al. [26] propose the designation of species for a selection of *Wolbachia* lineages. While this study considers the largest number of *Wolbachia* strains in genomic analyses to date, there are several shortcomings and oversights, which render the conclusions problematic. The authors state that *Wolbachia* taxonomy has been understudied, and they disregard the caution implemented in other phylogenetic and phylogenomic studies to not define species [5,10,13,22]. As an obligate intracellular symbiont, *Wolbachia* is intimately connected to its host. For this reason, and because *Wolbachia* cannot be cultured axenically, host phenotypes and phylogenetic relationships of *Wolbachia* strains are the most well studied aspects of *Wolbachia* biology rather than *Wolbachia* themselves. The strain designations used across the *Wolbachia* literature provide information on the host animal (ex: strain *wMel* infects *Drosophila melanogaster*), allowing results to be interpreted in the appropriate biological context. Below we outline three major

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concerns with the framework proposed by Ramirez-Puebla et al., and argue that applying species status to *Wolbachia* lineages is premature.

First, the datasets used by the authors are unsound. While the authors have gathered a large amount of the publically available *Wolbachia* genomes, sequence data known to be unreliable was used in their analyses. For example, the sequences available for the *wAna* strain (from *Drosophila ananassae*) represent a combination of host genomic inserts of *Wolbachia* from lateral gene transfer events and sequence from the *Wolbachia* infection [18]. Additionally, for genome strains *wDacA* and *wDacB* (from *Dactylopius coccus*), the authors do not provide primary sequence data, instead stating that their analysis will be published at a later time. Defining species status should be held to higher standards and publicly available datasets that the community can evaluate. Ramirez-Puebla et al. present low *in silico* DNA–DNA hybridization (DDH) and average nucleotide identity (ANI) values comparing *wDacA* to other A supergroup strains. Because the authors sequenced *wDacA* co-infecting with *wDacB* (a B supergroup *Wolbachia* strain), the data produced may be a combination of both strains or potential lateral gene transfer events, as often happens with *Wolbachia* [6,10,18,24]. The evidence for phage and chromosomal genetic exchange across supergroups between co-infecting strains further complicates species designations [2,8,23], though we appreciate that some *Wolbachia* supergroups evolve cohesively to a degree despite frequent ecological overlap in coinfecting hosts [10].

Second, and as previous studies recognized [5,13], the stability of any systematic framework is fundamentally dependent on data completeness for each candidate taxonomic grouping. As is evident by several recent studies that have identified novel *Wolbachia* supergroups, the field is still discovering new *Wolbachia* diversity [1,4,14,19]. Currently, there are 16 reported supergroups, denoted A–Q [1,4,14,15,21,27]. Supergroup G was de-commissioned as it was based on a recombinant from supergroups A and B [3]. Ramirez-Puebla et al. acknowledge the large number of *Wolbachia* supergroups, but only examine genomic data from strains representing six of these supergroups (A–F). They suggest that the name “*Wolbachia pipientis*” applies only to supergroup B, and propose new species names for seven other clades. This system of nomenclature would leave a total of ten supergroups nameless (supergroups H–Q), and it is unclear how these groups would be incorporated into their framework in future. Importantly, for two supergroups (C and D), there is a severely limited quantity of genomic data; two of the proposed species (*Candidatus Wolbachia blaxteri* and *Candidatus Wolbachia taylora*) are each based on data from a single *Wolbachia* strain. This should deter biologists from splitting each supergroup into two species (*Candidatus Wolbachia onchocercicola* and *Candidatus Wolbachia blaxteri* for supergroup C, and *Candidatus Wolbachia brugii* and *Candidatus Wolbachia taylora* for supergroup D). It is not clear how remaining members of the C and D supergroups not analyzed by Ramirez-Puebla et al. are to be classified. This could be especially problematic for strains that are found to cluster closely with, for example, supergroup D, but are sister to the clade of *Candidatus Wolbachia brugii* + *Candidatus Wolbachia taylora*.

Third, the utility of new species designations is not evident to us and presumably other researchers in the *Wolbachia* community. A combination of both genetic distance and functional divergence should be taken into consideration when devising taxonomic

frameworks. For example, there are many closely related *Wolbachia* strains in supergroup A (referred to as *Candidatus Wolbachia bourtzisii* in Ramírez-Puebla et al.) that infect *Drosophila* spp. and have vastly different consequences for their hosts [16]. Also, the proposed nomenclature could easily cause confusion. While a transitional process is expected when adopting new nomenclature, several of the suggested species names provide confusing descriptors for the clades. The name suggested for supergroup E is “*Candidatus Wolbachia collemboicola*” due to the fact that this clade is found infecting Collembolan hosts. However, there is evidence that this clade is not specific to Collembola, and can infect Dipteran species as well (J. Rasgon, unpublished data). The clade that would make up *Candidatus Wolbachia onchocercicola* (a subset of the C supergroup) includes *Wolbachia* strains that do not infect the genus *Onchocerca* [11]. However, all hosts of both the C and D supergroup strains are members of the Onchocercidae family.

The authors reference Pfarr et al. [25] as a source of support for splitting *Wolbachia* into species. Given the sequencing of additional genomes, discovery of many new supergroups, and discovery of novel phenotypes since then, the arguments made in Pfarr et al. are no longer as strong. Pfarr et al. argue that the nematode-infecting *Wolbachia* should be classified differently because they are obligate, infect a different taxon of hosts, and have genomes with different features. However, we now know of obligate *Wolbachia* strains infecting various arthropods [9,17,20,28,29], the F supergroup that infects both arthropods and nematodes [7], and that the *Wolbachia* infecting *Trichogramma* wasps share some similarities with the nematode-infecting strains, such as the absence of phages [12]. It is clear that *Wolbachia* do not “bin” nicely according to the criteria in Pfarr et al.

It is well understood that the *Wolbachia* clade is diverse and contains a number of distinct lineages identifiable to the community in the current supergroup classification system. Therefore, for these reasons and those above, we recommend to the community that they continue to use the previously designated strain names and supergroups for clarity in the field. Should there be further evidence and community support for species designations within this unique clade of bacteria, a comprehensive and voluntary *Wolbachia* consortium should work together to propose nomenclature, as has been previously done in the field (International *Wolbachia* Conference, Heron Island, Australia, August 2004).

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## References

1. Augustinos AA, Santos-Garcia D, Dionyssopoulou E, Moreira M, Papapanagiotou A, Scarvelakis M, Doudoumis V, Ramos S, Aguiar AF, Borges PAV, Khadem M, Latorre A, Tsiamis G, Bourtzis K. Detection and characterization of *Wolbachia* infections in natural populations of aphids: Is the hidden diversity fully unraveled? PLoS ONE. 2011; 6:e28695. [PubMed: 22174869]
2. Baldo L, Bordenstein S, Wernegreen JJ, Werren JH. Widespread recombination throughout *Wolbachia* genomes. Mol Biol Evol. 2006; 23:437–449. [PubMed: 16267140]
3. Baldo L, Werren JH. Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. Curr Microbiol. 2007; 55:81–87. [PubMed: 17551786]

4. Bing XL, Xia WQ, Gui JD, Yan GH, Wang XW, Liu SS. Diversity and evolution of the *Wolbachia* endosymbionts of *Bemisia* (Hemiptera: Aleyrodidae) whiteflies. *Ecol Evol.* 2014; 4:2714–2737. [PubMed: 25077022]
5. Bordenstein SR, Paraskevopoulos C, Dunning Hotopp JC, Sapountzis P, Lo N, Bandi C, Tettelin H, Werren JH, Bourtzis K. Parasitism and mutualism in *Wolbachia*: what the phylogenomic trees can and cannot say. *Mol Biol Evol.* 2009; 26:231–241. [PubMed: 18974066]
6. Brelsfoard C, Tsiamis G, Falchetto M, Gomulski LM, Telleria E, Alam U, Doudoumis V, Scolari F, Benoit JB, Swain M, Takac P, Malacrida AR, Bourtzis K, Aksoy S. Presence of extensive *Wolbachia* symbiont insertions discovered in the genome of its host *Glossina morsitans morsitans*. *PLoS Negl Trop Dis.* 2014; 8:e2728. [PubMed: 24763283]
7. Casiraghi M, Bordenstein SR, Baldo L, Lo N, Beninati T, Wernegreen JJ, Werren JH, Bandi C. Phylogeny of *Wolbachia pipientis* based on *gltA*, *groEL* and *ftsZ* gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. *Microbiology.* 2005; 151:4015–4022. [PubMed: 16339946]
8. Chafee ME, Funk DJ, Harrison RG, Bordenstein SR. Lateral phage transfer in obligate intracellular bacteria (*Wolbachia*): verification from natural populations. *Mol Biol Evol.* 2010; 27:501–505. [PubMed: 19906794]
9. Dedeine F, Vavre F, Fleury F, Loppin B, Hochberg ME, Bouletreau M. Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc Natl Acad Sci.* 2001; 98:6247–6252. [PubMed: 11353833]
10. Ellegaard KM, Klasson L, Naslund K, Bourtzis K, Andersson SGE. Comparative genomics of *Wolbachia* and the bacterial species concept. *PLoS Genet.* 2013; 9:e1003381. [PubMed: 23593012]
11. Ferri E, Bain O, Barbuto M, Martin C, Lo N, Uni S, Landmann F, Baccei SG, Guerrero R, de Souza Lima S, Bandi C, Wanji S, Diagne M, Casiraghi M. New insights into the evolution of *Wolbachia* infections in filarial nematodes inferred from a large range of screened species. *PLoS ONE.* 2011; 6:e20843. [PubMed: 21731626]
12. Gavotte L, Henri H, Stouthamer R, Charif D, Charlat S, Bouletreau M, Vavre F. A survey of the bacteriophage WO in the endosymbiotic bacteria *Wolbachia*. *Mol Biol Evol.* 2007; 24:427–435. [PubMed: 17095536]
13. Gerth M, Gansauge MT, Weigert A, Bleidorn C. Phylogenomic analyses uncover origin and spread of the *Wolbachia* pandemic. *Nat Commun.* 2014; 5:5117. [PubMed: 25283608]
14. Glowska E, Dragun-Damian A, Dabert M, Gerth M. New *Wolbachia* supergroups detected in quill mites (Acari: Syringophilidae). *Infect Genet Evol.* 2015; 30:140–146. [PubMed: 25541519]
15. Haegeman A, Vanholme B, Jacob J, Vandekerckhove TTM, Claeys M, Borgonie G, Gheysen G. An endosymbiotic bacterium in a plant-parasitic nematode: member of a new *Wolbachia* supergroup. *Int J Parasitol.* 2009; 39:1045–1054. [PubMed: 19504759]
16. Hoffmann AA, Ross PA, Rasic G. *Wolbachia* strains for disease control: ecological and evolutionary considerations. *Ecol Evol.* 2015; 8:751–768.
17. Hosokawa T, Koga R, Kikuchi Y, Meng XY, Fukatsu T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci.* 2010; 107:769–774. [PubMed: 20080750]
18. Hotopp JCD, Clark ME, Oliveira D, Foster JM, Fischer P, Torres MC, Giebel JD, Kumar N, Ishmael N, Wang SL, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science.* 2007; 317:1753–1756. [PubMed: 17761848]
19. Konecka E, Olszanowski Z. A screen of maternally inherited microbial endosymbionts in oribatid mites (Acari: Oribatida). *Microbiology.* 2015; 161:1561–1571. [PubMed: 25991706]
20. Kremer N, Charif D, Henri H, Bataille M, Prevost G, Kraaijeveld K, Vavre F. A new case of *Wolbachia* dependence in the genus *Asobara*: Evidence for parthenogenesis induction in *Asobara japonica*. *Heredity.* 2009; 103:248–256. [PubMed: 19513092]
21. Lo N, Casiraghi M, Salati E, Bazzocchi C, Bandi C. How many *Wolbachia* supergroups exist? *Mol Biol Evol.* 2002; 19:341–346. [PubMed: 11861893]

22. Lo N, Paraskevopoulos C, Bourtzis K, O'Neill SL, Werren JH, Bordenstein SR, Bandi C. Taxonomic status of the intracellular bacterium *Wolbachia pipientis*. *Int J Syst Evol Microbiol*. 2007; 57:654–657. [PubMed: 17329802]
23. Metcalf JA, Bordenstein SR. The complexity of virus systems: the case of endosymbionts. *Curr Opin Microbiol*. 2012; 15:546–552. [PubMed: 22609369]
24. Nikoh N, Tanaka K, Shibata F, Kondo N, Hizume M, Shimada M, Fukatsu T. *Wolbachia* genome integrated in an insect chromosome: evolution and fate of laterally transferred endosymbiont genes. *Genome Res*. 2008; 18:272–280. [PubMed: 18073380]
25. Pfarr K, Foster J, Slatko B, Hoerauf A, Eisen JA. On the taxonomic status of the intracellular bacterium *Wolbachia pipientis*: should this species name include the intracellular bacteria of filarial nematodes? *Int J Syst Evol Microbiol*. 2007; 57:1677–1678. [PubMed: 17684235]
26. Ramirez-Puebla ST, Servin-Garciduenas LE, Ormeno-Orrillo E, Vera-Ponce de Leon A, Rosenblueth M, Delaye L, Martinez J, Martinez-Romero E. 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. *Syst Appl Microbiol*. 2015; 38:390–399. [PubMed: 26189661]
27. Ros VID, Fleming VM, Feil EJ, Breeuwer JAJ. How diverse is the genus *Wolbachia*? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). *Appl Environ Microbiol*. 2009; 75:1036–1043. [PubMed: 19098217]
28. Russell JE, Stouthamer R. The genetics and evolution of obligate reproductive parasitism in *Trichogramma pretiosum* infected with parthenogenesis-inducing *Wolbachia*. *Heredity*. 2011; 106:58–67. [PubMed: 20442735]
29. Stouthamer R, Russell JE, Vavre F, Nunney L. Intragenomic conflict in populations infected by Parthenogenesis Inducing *Wolbachia* ends with irreversible loss of sexual reproduction. *BMC Evol Biol*. 2010; 10:12.