BIN overlap confirms transcontinental distribution of pest aphids (Hemiptera: Aphididae)

Authors: Muhammad Tayyib Naseem[1,2], Muhammad Ashfaq[3], Arif Muhammad Khan[1,4], Akhtar Rasool[1,5], Muhammad Asif[1], Paul D.N. Hebert[3]

Affiliations:

[1] National institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

[2] Pakistan Institute for Engineering and Applied Sciences, Islamabad, Pakistan

[3] Centre for Biodiversity Genomics & Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

[4] Department of Biotechnology, University of Sargodha, Sargodha, Pakistan

[5] Department of Zoology, University of Swat, Swat, Pakistan

Corresponding author:

Muhammad Ashfaq

Centre for Biodiversity Genomics & Department of Integrative Biology, University of Guelph, Guelph, ON, N1G 2W1, Canada

Email: mashfaq@uoguelph.ca

Phone: (519) 824-4120 Ext. 56393

Abstract:

DNA barcoding is highly effective for identifying specimens once a reference sequence library is available for the species assemblage targeted for analysis. Despite the great need for an improved capacity to identify the insect pests of crops, the use of DNA barcoding is constrained by the lack of a well-parameterized reference library. The current study begins to address this limitation by developing a DNA barcode reference library for the pest aphids of Pakistan. It also examines the affinities of these taxa with conspecific taxa from other geographic regions based on both conventional taxonomy and Barcode Index Numbers (BINs). A total of 809 aphids were collected from 123 plant species at 87 sites across Pakistan. Morphological study and DNA barcoding allowed 774 specimens to be identified to one of 42 species while the others were placed to a genus or subfamily. The 801 sequences obtained from these specimens were assigned to 52 BINs whose monophyly were supported by neighbor-joining (NJ) clustering and Bayesian inference. The 42 species were assigned to 41 BINs with 38 showing BIN concordance; one species (*Rhopalosiphum padi*) was assigned to two BINs, while two others (*Aphis affinis, Aphis gossypii*) were assigned to the same BIN, while one species (*Aphis astragalina*) lacked a qualifying sequence. The 42 Linnaean species were represented on BOLD by 7,870 records from 69 countries. Combining these records with those from Pakistan produced to 60 BINs with 12 species showing a BIN split and three a BIN merger. Geo-distance correlations showed that intraspecific divergence values for 18 of 37 species were not affected by the distance between

37 populations. Forty four of the 52 BINs from Pakistan had counterparts in 73 countries across six
38 continents, documenting the broad distributions of pest aphids.

39 Key words: Hemiptera, plant-pest, DNA barcoding, Barcode Index Number, COI, Pakistan

Introduction

Although aphids (Hemiptera: Aphididae) are important plant pests, their life stage diversity and phenotypic plasticity have constrained the development of effective taxonomic keys [1,2]. With over 4,700 described species, the Aphididae is the largest family within the Aphidoidea [3]. Most pest aphids belong to the subtribe Aphidina which includes 670 described species [3,4]. Nearly 100 aphid species have been listed as serious agricultural pests; they attack more than 300 plant species [5,6], and lower crop yield by direct feeding and by transmitting viral diseases [7].

Sibling species complexes are common in many pest aphids [8]. Very often, these species are morphologically identical but genetically distinct [9]. They often include anholocyclic biotypes (=clones) with differing host preferences and varying competency for disease transmission [10,11]. Species identification is so challenging that taxonomic keys are either ineffective or only useful for a particular geographic area or taxonomic group 12]. These deficits have prompted the search for alternative approaches for identification such as cybertaxonomy [13] and DNA sequencing [14,15]. However, the later approach has gained stronger uptake due to its universal applicability, low cost, and strong performance [16].

Past studies have shown that DNA-based approaches can enable both specimen identification and the clarification of cryptic species complexes [17,18]. Diverse mitochondrial and nuclear genes have been used individually and in combination to discriminate insect species [19-21]. Although multigene analysis is valuable in resolving complex taxonomic situations and is essential for phylogenetic reconstructions [22,23], it has seen little adoption for routine identifications [18]. By contrast, DNA barcoding [24] employs a 658 bp segment of a single

gene, cytochrome *c* oxidase I, to discriminate animal species. Because of its ease of application and low cost, DNA barcoding has gained broad uptake [25-28]. It is now commonly used to identify specimens and to resolve sibling species complexes in insects including aphids [29-32].

The application of DNA barcoding requires bioinformatics support and a well-parameterized reference library [33]. The Barcode of Life Data System (BOLD – www.boldsystems.org) [34] meets the former need and currently includes more than six million barcode records from animals. Most of these records are from insects (5.2 million) and 49,000 records derive from aphids (accessed 3 July 2019). All barcode sequences meeting quality criteria receive a Barcode Index Number (BIN) [35]. BINs are an effective species proxy because they correspond closely with species designated through morphological study [36,37]. As a result, BINs are now routinely employed for biodiversity assessments, counting species, analyzing cryptic species complexes, and assessing species ranges [38-40]. These developments have generated a high level of interest in DNA barcoding, leading to reference barcode libraries for certain groups at continental and global scales [32,41-45].

Although the DNA barcode library for insects is incomplete, it is already valuable for identifying pest species and assessing their distributions [29,42,46-49]. However, the lack of reference sequences constrains the utility of DNA barcoding in many situations. Although barcode coverage for the aphid fauna of some countries is extensive [43,50,51] work in other nations, including Pakistan, has been limited. The current study addresses this gap by generating a barcode reference library for the pest aphids of Pakistan, and by using BINs to reveal their links to aphid assemblages in other regions.

Materials and Methods

Ethics Statement

No specific permissions were required for this study. The study did not involve endangered or protected species.

Aphids were sampled from 123 plant species representing 33 families at 87 sites in Pakistan (Fig. 1, S1 Table) during 2010-2013. These sites included agricultural settings, nurseries, national parks, botanical gardens, natural forests, and disturbed habitats. Based on GPS coordinates, the collection sites were rendered using SimpleMappr.net (Fig. 1). Aphids were collected by either beating foliage above a white paper sheet or by removing them from their host plant with a camel hair brush [52]. Collections were transferred into Eppendorf tubes prefilled with 95% ethanol and stored at -20°C until analysis.

Identification

Aphids were identified using standard taxonomic keys [52,53]. Morphological characters were examined with a Labomed CZM6 stereomicroscope (Labo America) equipped with an ocular micrometer. Each specimen was assigned to a Linnaean species based on morphology and the identification was later validated by DNA barcode sequence matches on BOLD.

DNA Barcoding

Front-end processing, including specimen sorting, arraying, databasing, and imaging was performed at the Insect Molecular Biology Laboratory, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad. Individual specimens were placed into 96-well format in a microplate pre-filled with 30 µl of 95% ethanol in each well. Each specimen was photographed dorsally using a 12 megapixel Olympus µ-9000 camera (Olympus America Inc., USA) mounted on a stereomicroscope. Specimen metadata (collection information and taxonomy) and images were submitted to BOLD under the project MAAPH, "Barcoding Aphid Species of Pakistan". DNA extraction, PCR amplification, and sequencing were carried out at Centre for Biodiversity Genomics at Guelph. DNA extraction followed Ivanova et al. [54] with voucher recovery protocol [55]. PCR amplification of the COI-5′ (barcode region) [24] was performed using primer pair C_LepFolF (forward) and C_LepFolR (reverse) (http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_PrimerSets.pdf) in 12.5 µL reactions that included standard PCR ingredients [56] and 2 µL of DNA template. The thermocycling regime was: 94°C (1 min), 5 cycles at 94°C (40 s), 45°C (40 s), 72°C (1 min); 35 cycles at 94°C (40 s), 51°C (40 s), 72°C (1 min); and a final extension at 72°C (5 min). PCR success was verified by analyzing the amplicons on 2% agarose E-gel® 96 system (Invitrogen Inc.). Specimens which failed to amplify in the first round of PCR were re-run with primers LepF2_t1 (TGTAAAACGACGGCCAGTAATCATAARGATATYGG) [57] and LepR1 using the same PCR conditions. PCR products were sequenced bidirectionally on an Applied Biosystems 3730XL DNA Analyzer using the BigDye Terminator Cycle Sequencing Kit (v3.1) (Applied Biosystems). Sequences were edited using CodonCode Aligner (CodonCode Corporation, USA),

and translated on MEGA v6 [58] to confirm they were free of stop codons, and submitted to BOLD. The specimen metadata and sequences generated in this study are available on BOLD in the dataset DS-MAAPH.

Data Analysis

All barcode sequences were compared with those on BOLD and GenBank to ascertain sequence similarities. Sequence matches on BOLD were obtained using "Identification Engine" while nBLAST (http://www.ncbi.nlm.nih.gov/blast/) was used on GenBank. All sequences meeting quality standards (>500 bp, <1% ambiguous bases, no stop codon or contamination flag) were assigned to a BIN [35]. BIN discordance and BIN overlap reports were generated using analytical tools on BOLD. As a test of the reliability of species discrimination, the presence or absence of a 'barcode gap' [59] among species was determined on BOLD. A species was considered distinct when its maximum intraspecific distance was less than the distance to its nearest neighbor (NN).

Nucleotide alignments and neighbor-joining (NJ) analysis [60] were conducted in MEGA6. The NJ analysis employed the Kimura-2-Parameter (K2P) [61] distance model, with pairwise deletion of missing sites, and 1000 bootstrap replicates for the nodal support. Bayesian inference was performed in MrBayes v3.2.0 [62] employing the Markov Chain Monte Carlo (MCMC) technique. This analysis was based on representative sequences from 67 aphid haplotypes in the dataset extracted using DNaSP v5.10 [63] with *Diaphorina citri* (Hemiptera: Psyllidae) as outgroup. The data were partitioned in two ways; i) a single partition with parameters estimated across all codon positions, ii) a codon-partition in which each codon position was allowed

142 different parameter estimates. The evolution of sequences was modelled by the GTR+Γ model
143 independently for the two partitions using the ‘‘unlink’’ command in MrBayes, and the best
144 model was selected using FindModel (www.hiv.lanl.gov/cgi-bin/findmodel/findmodel.cgi). The
145 analyses were run for 10 million generations using four chains with sampling every 1000
146 generations. Bayesian posterior probabilities were calculated from the sample points once the
147 MCMC algorithm converged. Convergence was defined as the point where the standard
148 deviation of split frequencies was less than 0.002 and the PSRF (potential scale reduction factor)
149 approached 1, and both runs converged to a stationary distribution after the burn-in (by default,
150 the first 25% of samples were discarded). Each run produced 100001 samples of which 75001
151 samples were included. The trees generated through this process were visualized using FigTree
152 v1.4.0.

153 BOLD was searched for barcode records for the 42 Linnaean species encountered in this study.
154 The resultant records were examined for BIN assignment [35] and used in a geo-distance
155 correlation analysis to examine the relationship between geographic and genetic distance in each
156 species. Two methods were employed in the latter analysis; the Mantel Test [64] was used to
157 examine the relationship between geographic (km) and genetic (K2P) distance matrices, while
158 the second approach compared the spread of the minimum spanning tree of collection sites and
159 maximum intra-specific divergence [65]. The relationship between geographic and intraspecific
160 distances was analyzed for each species with at least one individual from three or more sites.
161 BINs recovered from Pakistan were also used in BIN-overlap analysis on BOLD to ascertain the
162 incidence of unique BINs in a region, and to estimate overlap in BIN composition.

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Results

Morphological analysis facilitated by the barcode data allowed 774 of the 809 specimens to be identified to 42 species, each an important crop pest (S1 Table). Another 32 specimens could be placed to a genus while the other three could only be assigned to a subfamily (Aphidinae). Overall, the 809 specimens included representatives of 30 genera, five subfamilies (Aphidinae, Calaphidinae, Chaitophorinae, Eriosomatinae, Lachninae) of the Aphididae (S2 Table). Members of the Aphidinae were dominant (n=780) as the other four subfamilies were represented by just 29 specimens with Chaitophorinae and Lachninae each contributing one specimen (S2 Table). Among the genera, *Aphis* was most common (n=306), and it was represented by eight identified and three undetermined species. *Myzus* was the second most frequent genus (n=170), but it was only represented by one species, *Myzus persicae*. *Rhopalosiphum,* the third most abundant (83) genus, was represented by three major pest species (*R. maidis, R. padi, R. rufiabdominale*). Two species (*Aphis astragalina, Periphyllus lyropictus*) represented first records for Pakistan while two others (*Lipaphis pseudobrassicae, Sarucallis kahawaluokalani*) were known, but were recorded as *Lipaphis erysimi* and *Tinocallis kahawaluokalani*, names now relegated to synonymy.

The 809 barcode sequences provided two or more records for 36 of the 42 species and single records for the rest (Table 1, S1). Maximum K2P divergence values within species ranged from 0 – 3.6% (mean=0.1%), while the within genus values were 0.8 – 10.3% (mean=7.4%), and within family (Aphididae) 3.7 – 17.3% (mean=9.6%) (Table 1). Barcode gap analysis examined the ability of barcodes to discriminate the 42 named species. With the exception of one species

(*Aphis gossypii*), where the maximum intraspecific distance (3.6%) overlapped with *A. affinis*, the maximum intraspecific distance for each species was less than its NN distance (Fig. 2A + Fig. 2B). This pattern did not change with increased sample size (Fig. 2C).

Nearly all sequences (801/809) qualified for a BIN assignment, and they were placed in 52 BINs. The 774 specimens of the 42 Linnaean species were assigned to 41 BINs; 38 showed BIN concordance (species members = BIN members), one species (*Rhopalosiphum padi*) was split (AAA9899, ACF2924), and two species (*Aphis affinis, A. gossypii*) were merged (AAA3070), while another (*Aphis astragalina*) lacked a BIN assignment due to its low quality sequence (410 bp, 9 Ns). The 32 specimens lacking a species assignment were placed in 9 BINs – three for *Aphis* and one for each of the other six genera (*Acyrthosiphon, Capitophorus, Forda, Hyalopterus, Macrosiphoniella, Schizaphis*). The three specimens only identified to a subfamily were assigned to two BINs. NJ analysis (Fig. 3) and Bayesian inference (BI) (Fig. 4) supported the monophyly of each of the 52 BINs. The NJ and BI also discriminated the species or genera that either lacked (*Aphis astragalina*) or shared BINs (*Aphis gossypii*, *A. affinis*), as they formed distinct branches on the NJ and BI trees (Fig. 3, Fig. 4).

Geo-distance correlation analysis for 37 species was conducted by including 5,067 sequences from conspecific individuals on BOLD. This analysis showed that intraspecific divergence in 49% of the species was not affected by expanding analysis to consider its entire range (Mantel test, $P>0.01$) (Table 2). The other 51%, that were affected by geographic range, included six species with BIN splits and eight with intraspecific divergence higher than >2%. The distributional patterns of aphids detected in Pakistan were further analyzed by examining BIN overlap between Pakistan and other countries, a comparison that involved 9,905 barcode records

assigned to the 52 BINs. This analysis showed that 27 of the 52 BINs were recorded from four or more continents while eight were unique to Pakistan (Table 3). Except for *Acyrthosiphon malvae* and *Uroleucon sonchi*, all named species (40) analyzed in this study already had barcode records from multiple countries and continents (Table 3).

Discussion

Prior morphological surveys on the aphids of Pakistan have reported the presence of nearly 300 species [66-68]. Most of this work focused on specific geographic regions [69] or on species attacking crops [70,71]. The current study surveyed aphids across much of Pakistan from a wider range of host plants, but primarily aimed to develop a barcode reference library for the fauna. Prior studies have begun to construct barcode reference libraries for some pest insect groups, such as aphids in Canada [29], leafminers in USA [72], fruit flies in Africa [42], food pests in Korea [46], thrips in Pakistan [28], looper moths in British Columbia [73], and mealybugs in China [49]. These libraries have stimulated the use of DNA barcoding in biosecurity and plant protection programs [74], but this use have revealed the need for expanded parameterization of the libraries to improve their utility in diagnosing newly encountered species. Barcode libraries for two major pest insect groups in Pakistan, thrips and whiteflies, are well advanced [28,75], but other groups have seen little attention in this country.

Most aphids analyzed in this study could be assigned to a species, but 35 specimens could only be resolved to a genus or subfamily. In part, this difficulty reflected the fact that many important

227 pest aphids are cryptic species complexes whose members are almost impossible to discriminate
228 morphologically [39]. For example, *Aphis gossypii* is a particularly challenging species complex
229 [5,13]; it includes at least 20 morphologically indistinguishable species [76] likely explaining its
230 wide range of primary and secondary host plants [77]. In the present study, DNA barcoding
231 separated all eight species of the genus *Aphis* that were encountered. Although K2P distances
232 between two species pairs; i) *A. affinis* and *A. gossypii* (1.4%), ii) *A. astragalina* and *A.*
233 *craccivora* (0.8%) were low, both NJ analysis and Bayesian inference supported the monophyly
234 of each species. The COI divergences in this study are similar to those reported in prior
235 investigations [29,78,79] which reported low sequence divergence between sibling species such
236 as *A. gossypii* and *A. affinis* [29].

237 Prior studies have shown strong correspondence between BINs and known species [36],
238 especially when reference specimens are identified by experts [80]. The same pattern was
239 apparent in this study as 38 of 41 Linnaean species were assigned to a single BIN. There were
240 only two exceptions; *R. padi* was assigned to two BINs and *A. gossypii – A. affinis* were assigned
241 to the same BIN. By comparison, when barcode sequences from conspecific specimens from
242 other countries were considered, 12 of the 4 species showed a BIN split, an outcome which likely
243 indicates incorrectly identified specimens [36]. Interestingly, the BIN (AAA3070) shared by
244 specimens of *A. gossypii* and *A. affinis* from Pakistan included 31 additional species names when
245 all records for it on BOLD were considered. Misidentifications and overlooked cryptic species
246 may often cause conflicts between BIN and species morphology [81], but this can only be
247 resolved by detailed taxonomic studies [82].

Geo-distance correlations showed that the genetic divergence in almost half of the aphid species increased with geographic distance while the others were unimpacted. Interestingly, the inclusion of conspecific sequences from other regions also increased the incidence of BIN splits. Since these analyses included all the conspecific sequences on BOLD, this outcome may reflect taxonomic errors [83]. Although spatial variation in conspecific sequences sometimes leads to increased intraspecific divergence values [84], it is usually too low to reduce the capacity of DNA barcodes to deliver reliable species identifications [44,85].

BINs are valuable in evaluating the geographic range of aphid species because they circumvent taxonomic uncertainties. BINs are gaining increased use to estimate species numbers [38] and to understand their distributions [49]. This analysis revealed that 27 of the 44 BINs with prior records on BOLD occurred on four or more continents, highlighting the broad ranges of many pest aphids. For example, BINs for *Aphis fabae* (black bean aphid), *A. nerii* (oleander aphid), *A. craccivora* (groundnut aphid), *Acyrthosiphon pisum* (pea aphid), *Brachycaudus helichrysi* (plum aphid), *Brevicoryne brassicae* (cabbage aphid), *L. pseudobrassicae* (turnip aphid), *R. padi* (oat aphid), *R. maidis* (corn aphid), *Macrosiphum euphorbiae* (potato aphid), *M. persicae* (peach aphid), and *Therioaphis trifolii* (alfalfa aphid) were all recorded from six continents. Interestingly, BINs associated with some of these species were also linked with other species on BOLD. For instance, AAA3070 was linked to 33 other species of *Aphis* while AAA6213 was associated with 13 species of *Macrosiphum*, and AAA5565 with nine species of *Aphis*. Although some of these cases may involve BIN sharing by different species [29], most cases likely reflect misidentifications.

269 The level of BIN overlap between the aphid fauna of Pakistan is much higher (85%) than levels
270 for moths (44%) [86] and spiders (24%) [87]. This difference, may, be due, in part, to the fact
271 that the winged alates of aphids can disperse long distances are they use widely crop plants as
272 their hosts [88]. Consequently, the number of aphid species known from Europe has increased by
273 20% in the last 30 years [89] reflecting their transport on produce fruits [49], coupled with
274 shifting environmental regimes. Reports suggest that with every 1°C increase, some 15
275 additional aphid species were recorded in Europe [90]. In North America, about 18% of all aphid
276 species are introduced, and nearly half are plant pests [91]. Rapid developments in DNA
277 sequencing are enabling the documentation of pest species and their distribution across the globe,
278 but conflicts between taxonomic assignments and sequences have limited the full utility of these
279 data. Given this difficulty, the BIN system provides an alternative path to document and track the
280 pest species on a planetary scale.

281

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References:

1. Braendle C, Davis GK, Brisson JA, Stern DL. Wing dimorphism in aphids. Heredity. 2006;97: 192.
2. Ogawa K, Miura T. Aphid polyphenisms: trans-generational developmental regulation through viviparity. Front Physiol. 2014;5: 1.
3. Favret C. Aphid species file version 5.0 2017 [Available from: http://aphid.speciesfile.org/].
4. Blackman R, Eastop V. Aphids on the world's plants: An online identification and information guide; 2014.
5. Blackman RL, Eastop VF. Taxonomic issues. In: Emden H F van, Harrington R, editors. Aphids as crop pests: CAB International; 2007. pp. 1–27.
6. Dixon AFG, Kindlmann P, Leps J, Holman J. Why there are so few species of aphids especially in the tropics. Am Nat. 1987;129: 580–592.
7. Sewell GH, Storch RH, Manzer FE, Forsythe HY Jr. The relationship between coccinellids and aphids in the spread of potato leafroll virus in a greenhouse. Am J Potato Res. 1990;67: 865–868.
8. Mosco MC, Arduino P, Bullini L, Barbagallo S. Genetic heterogeneity, reproductive isolation and host preferences in mealy aphids of the *Hyalopterus pruni* complex (Homoptera, Aphidoidea). Mol. Ecol. 1997; 6: 667–670.
9. Carletto J, Blin A, Vanlerberghe-Masutti F. DNA-based discrimination between the sibling species *Aphis gossypi*i Glover and *Aphis frangulae* Kaltenbach. Syst Entomol. 2009;34: 307–314.

10. Sorensen JT. Aphids In: Cardé RT, Resh VH, editors. Encyclopedia of insects, 2nd edition. San Diego, USA: Academic Press; 2009. p. 27–31.
11. Raymond B, Searle JB, Douglas AE. On the processes shaping reproductive isolation in aphids of the *Aphis fabae* (scop.) complex (Aphididae: Homoptera). Biol J Linnean Soc. 2001;74: 205–215.
12. Brisson JA. Aphid wing dimorphisms: linking environmental and genetic control of trait variation. Philosophical Transactions of the Royal Society of London Series B, Biol Sci. 2010;365(1540): 605–16.
13. Favret C. Cybertaxonomy to accomplish big things in aphid systematics. Insect Sci. 2014;21: 392–399.
14. Naaum AM, Foottit RG, Maw HEL, Hanner R. Real-time PCR for identification of the soybean aphid, *Aphis glycines* Matsumura. J. Appl. Entomol. 2014;138: 485–9.
15. Kinyanjui G, Khamis FM, Mohamed S, Ombura LO, Warigia M, Ekesi S. Identification of aphid (Hemiptera: Aphididae) species of economic importance in Kenya using DNA barcodes and PCR-RFLP-based approach. Bull Entomol Res. 2016;106: 63–72.
16. Nunez JCB, Oleksiak MF. A cost-effective approach to sequence hundreds of complete mitochondrial genomes. PLOS ONE. 2016;11: e0160958.
17. Caterino MS, Cho S, Sperling FA. The current state of insect molecular systematics: A thriving Tower of Babel. Annu Rev Entomol. 2000;45: 1–54.
18. Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, et al. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol. 2006;55: 595–609.

19. Erlandson M, Braun L, Baldwin D, Soroka J, Ashfaq M, Hegedus D. Molecular markers for *Peristenus* spp. (Hymenoptera: Braconidae) parasitoids associated with *Lygus* spp. (Hemiptera: Miridae). Can Entomol. 2003;135: 71–83.
20. Ashfaq M, Ara J, Noor AR, Hebert PDN, Mansoor S. Molecular phylogenetic analysis of a scale insect (*Drosicha mangiferae*; Hemiptera: Monophlebidae) infesting mango orchards in Pakistan. Eur J Entomol. 2011;108: 553–559.
21. Chen R, Jiang L-Y, Qiao G-X. The effectiveness of three regions in mitochondrial genome for aphid DNA barcoding: A case in Lachininae. PLOS ONE. 2012;7: e46190.
22. Ashfaq M, Noor AR, Mansoor S. DNA-based characterization of an invasive mealybug (Hemiptera: Pseudococcidae) species damaging cotton in Pakistan. Appl Entomol Zool. 2010;45: 395–404.
23. Liu J, Jiang J, Song S, Tornabene L, Chabarria R, Naylor GJP, et al. Multilocus DNA barcoding – species identification with multilocus data. Sci Rep. 2017;7: 16601.
24. Hebert PD, Cywinska A, Ball SL. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B: Biol Sci. 2003;270(1512): 313–321.
25. Hebert PD, Ratnasingham S, de Waard JR. Barcoding animal life: Cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B: Biol Sci. 2003;270(Suppl 1): S96–S99.
26. Ander M, Troell K, Chirico J. Barcoding of biting midges in the genus *Culicoides*: a tool for species determination. Med Vet Entomol. 2013;27: 323–31.
27. Adams M, Raadik TA, Burridge CP, Georges A. Global biodiversity assessment and hyper-cryptic species complexes: more than one species of elephant in the room? Syst Biol. 2014;63: 518–33.

28. Iftikhar R, Ashfaq M, Rasool A, Hebert PDN. DNA barcode analysis of thrips (Thysanoptera) diversity in Pakistan reveals cryptic species complexes. PLOS ONE. 2016;11: e0146014.

29. Foottit RG, Maw HEL, Von Dohlen CD, Hebert PDN. Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. Mol Ecol Resour. 2008;8: 1189–1201.

30. Liu Q-H, Jiang L-Y, Qiao G-X. DNA barcoding of Greenideinae (Hemiptera: Aphididae) with resolving taxonomy problems. Invertebr Syst. 2013;27: 428–438.

31. Cocuzza GE, Cavalieri V. Identification of aphids of *Aphis frangulae*-group living on Lamiaceae species through DNA barcode. Mol Ecol Resour. 2014;14: 447–57.

32. Gwiazdowski RA, Foottit RG, Maw HEL, Hebert PDN. The Hemiptera (Insecta) of Canada: constructing a reference library of DNA barcodes. PLOS ONE. 2015;10: e0125635.

33. Galimberti A, Ferri E, De Mattia F, Labra M, Casiraghi M. DNA barcoding: a six-question tour to improve users' awareness about the method. Brief Bioinform. 2010;11: 440–53.

34. Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Mol Ecol Notes. 2007;7: 355–364.

35. Ratnasingham S, Hebert PDN. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLOS ONE. 2013;8: e66213.

36. Mutanen M, Kekkonen M, Prosser SWJ, Hebert PDN, Kaila L. One species in eight: DNA barcodes from type specimens resolve a taxonomic quagmire. Mol Ecol Resour. 2015;15: 967–984.

37. Ortiz AS, Rubio RM, Guerrero JJ, Garre MJ, Serrano J, Hebert PDN, et al. Close congruence between Barcode Index Numbers (BINs) and species boundaries in the Erebidae (Lepidoptera: Noctuoidea) of the Iberian Peninsula. Biodivers data J. 2017;5: e19840.

38. Hebert PDN, Ratnasingham S, Zakharov EV, Telfer AC, Levesque-Beaudin V, Milton MA, et al. Counting animal species with DNA barcodes: Canadian insects. Phil Trans R Soc Lond B: Biol Sci. 2016; 371(1702).

39. Lee Y, Lee W, Kanturski M, Foottit RG, Akimoto SI, Lee S. Cryptic diversity of the subfamily Calaphidinae (Hemiptera: Aphididae) revealed by comprehensive DNA barcoding. PLOS ONE. 2017;12: e0176582.

40. Ashfaq M, Sabir JSM, El-Ansary HO, Perez K, Levesque-Beaudin V, Khan AM, et al. Insect diversity in the Saharo-Arabian region: Revealing a little-studied fauna by DNA barcoding. PLOS ONE. 2018;13: e0199965.

41. deWaard JR, Mitchell A, Keena MA, Gopurenko D, Boykin LM, Armstrong KF, et al. Towards a global barcode library for *Lymantria* (Lepidoptera: Lymantriinae) tussock moths of biosecurity concern. PLOS ONE. 2010;5: e14280.

42. Virgilio M, Jordaens K, Breman FC, Backeljau T, De Meyer M. Identifying insects with incomplete DNA barcode libraries, African fruit flies (Diptera: Tephritidae) as a test case. PLOS ONE. 2012;7: e31581.

43. Coeur d'Acier A, Cruaud A, Artige E, Genson G, Clamens AL, Pierre E, et al. DNA barcoding and the associated phylaphidb@se website for the identification of European aphids (Insecta: Hemiptera: Aphididae). PLOS ONE. 2014;9: e97620.

400 44. Ashfaq M, Akhtar S, Khan AM, Adamowicz SJ, Hebert PDN. DNA barcode analysis of
401 butterfly species from Pakistan points towards regional endemism. Mol Ecol Resour.
402 2013;13(5):832–843.
403 45. Raupach MJ, Hannig K, Moriniere J, Hendrich L. A DNA barcode library for ground
404 beetles of Germany: the genus *Amara* Bonelli, 1810 (Insecta, Coleoptera, Carabidae).
405 Zookeys. 2018;759: 57–80.
406 46. Cho SY, Suh KI, Bae YJ. DNA barcode library and its efficacy for identifying
407 food-associated insect pests in Korea. Entomol Res. 2013;43: 253–261.
408 47. Ashfaq M, Hebert PDN. DNA barcodes for bio-surveillance: Regulated and economically
409 important arthropod plant pests. Genome 2016;59: 933–945.
410 48. Kirichenkoa NI, Petkoa VM, Magnoux CE, Lopez-Vaamonde C. Diversity and
411 distribution of leaf mining insects on birches (*Betula* spp.) in Siberia. Entomol Rev.
412 2017;97: 183–197.
413 49. Ren J-M, Ashfaq M, Hu X-N, Ma J, Liang F, Hebert PDN, et al. Barcode Index Numbers
414 expedite quarantine inspections and aid the interception of nonindigenous mealybugs
415 (Pseudococcidae). Biol Invasions 2018;20: 449–460.
416 50. Foottit RG, Maw HEL, Pike KS. DNA barcodes to explore diversity in aphids
417 (Hemiptera Aphididae and Adelgidae. Redia 2009;XCII: 87–91.
418 51. Lee W, Kim H, Lim J, Choi HR, Kim Y, Kim YS, et al. Barcoding aphids (Hemiptera:
419 Aphididae) of the Korean Peninsula: Updating the global data set. Mol Ecol Resour.
420 2011;11: 32–37.
421 52. Blackman RL, Eastop VF. Aphids on the world's crop: An identification and information
422 guide, 2nd Edition. Wiley; 2000.

423 53. Martin JH. The identification of common aphid pests of tropical agriculture. Trop Pest
424 Manag. 1983;29: 395–411.
425 54. Ivanova NV, deWaard JR, Hebert PDN. An inexpensive, automation-friendly protocol
426 for recovering high quality DNA. Mol Ecol Notes 2006;6: 998–1002.
427 55. Porco D, Rougerie R, Deharveng L, Hebert P. Coupling non-destructive DNA extraction
428 and voucher retrieval for small soft-bodied arthropods in a high-throughput context: The
429 example of Collembola. Mol Ecol Resour. 2010;10: 942–945.
430 56. Hebert PDN, Dewaard JR, Zakharov EV, Prosser SW, Sones JE, McKeown JT, et al. A
431 DNA 'barcode blitz': rapid digitization and sequencing of a natural history collection.
432 PLOS ONE. 2013;8: e68535.
433 57. Park DS, Foottit R, Maw E, Hebert PDN. Barcoding bugs: DNA-based identification of
434 the true bugs (Insecta: Hemiptera: Heteroptera). PLOS ONE. 2011;6: e18749.
435 58. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary
436 genetics analysis version 6.0. Mol Biol Evol. 2013;30: 2725–2729.
437 59. Meyer CP, Paulay G. DNA barcoding: Error rates based on comprehensive sampling.
438 PLOS BIOL. 2005;3: e422.
439 60. Thompson JD, Higgins DG, Gibson TJ. Clustal W: Improving the sensitivity of
440 progressive multiple sequence alignment through sequence weighting, position-specific
441 gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22: 4673–4680.
442 61. Kimura M. A simple method for estimating evolutionary rates of base substitutions
443 through comparative studies of nucleotide sequences. J Mol Evol. 1980;16: 111–120.

444 62. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. Mrbayes
445 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model
446 space. Syst Biol. 2012;61: 539–542.
447 63. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA
448 polymorphism data. Bioinformatics 2009;25: 1451–1452.
449 64. Mantel N. The detection of disease clustering and a generalized regression approach.
450 Cancer Res. 1967;27: 209–220.
451 65. Blagoev GA, deWaard JR, Ratnasingham S, deWaard SL, Lu L, Robertson J, et al.
452 Untangling taxonomy: a DNA barcode reference library for Canadian spiders. Mol Ecol
453 Resour. 2016;16: 325–341.
454 66. Naumann-Etienne K, Remaudiere G. A commented preliminary checklist of the aphids
455 (Homoptera: Aphididae) of Pakistan and their host plants. Parasitica 1995;51: 61.
456 67. Irshad M. Aphids and their biological control in Pakistan. Pak J Biol Sci. 2001;4: 537–
457 541.
458 68. Kanturski M, Zia A, Rafi MA. The *Lachnus* of Pakistan with description of a new species
459 (Hemiptera: Aphididae: Lachninae). J Asia-Pac Entomol. 2017;20: 1219–1227.
460 69. Bodlah I, Naeem M, Mohsin A. Checklist distribution host range and ecology of
461 Aphidoidea (Homoptera) from the rainfed region of Punjab province of Pakistan, Sarhad
462 J Agric. 2011;27: 93–101.
463 70. Hamid S. Natural balance of graminicolous aphids in Pakistan. Survey of populations.
464 Agronomie 1983;3: 665–673.
465 71. Amin M, Mahmood K, Bodlah I. Aphid species (Hemiptera: Aphididae) infesting
466 medicinal and aromatic plants in the Poonch division of Azad Jammu and Kashmir,
467 Pakistan. J Animal Plant Sci. 2017;27: 1377–1385.

72. Scheffer SJ, Lewis ML, Gaimari SD, Reitz SR. Molecular survey for the invasive leafminer pest *Liriomyza huidobrensis* (Diptera: Agromyzidae) in California uncovers only the native pest *Liriomyza langei*. J Econ Entomol. 2014;107: 1959–64.

73. deWaard JR, Hebert PDN, Humble LM. A comprehensive DNA barcode library for the looper moths (Lepidoptera: Geometridae) of British Columbia, Canada. PLOS ONE. 2011;6: e18290.

74. Hodgetts J, Ostoja-Starzewski JC, Prior T, Lawson R, Hall J, Boonham N. DNA barcoding for biosecurity: case studies from the UK plant protection program. Genome. 2016;59: 1033–48.

75. Ashfaq M, Hebert PDN, Mirza MS, Khan AM, Mansoor S, Shah GS, et al. DNA barcoding of *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) reveals southerly expansion of the dominant whitefly species on cotton in Pakistan. 2014;PLOS ONE 9: e104485.

76. Stroyan HLG. Aphids – Pterocommatinae and Aphidinae (Aphidini). Homoptera, Aphididae: Roy Entomol Soc., UK; 1984;2: 232 pp.

77. Kring JB. Biological separation of *Aphis gossypii* Glover and *Aphis*: Sedi Kaltenbach. Ann Entomol Soc Am. 1955;48(6): 442–444.

78. Wang JF, Qiao GX. DNA barcoding of genus *Toxoptera* Koch (Hemiptera: Aphididae): Identification and molecular phylogeny inferred from mitochondrial COI sequences. Insect Sci. 2009;16: 475–484.

79. Cocuzza GE, Cavalieri V, Barbagallo S. Preliminary results in the taxonomy of the cryptic group *Aphis frangulae/gossypii* obtained from mitochondrial DNA sequence. B Insectol. 2008;61: 125–126.

80. Janzen DH, Burns JM, Cong Q, Hallwachs W, Dapkey T, Manjunath R, et al. Nuclear genomes distinguish cryptic species suggested by their DNA barcodes and ecology. Proc Natl Acad Sci USA. 2017. https://doi.org/10.1073/pnas.1621504114.

81. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc Natl Acad Sci USA. 2004;101: 14812–7.

82. Bortolus A. Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. Ambio. 2008;37: 114–8.

83. Virgilio M, Backeljau T, Nevado B, De Meyer M. Comparative performances of DNA barcoding across insect orders. BMC Bioinformatics. 2010;11: 206.

84. Bergsten J, Bilton DT, Fujisawa T, Elliott M, Monaghan MT, Balke M, et al. The effect of geographical scale of sampling on DNA barcoding. Syst Biol. 2012;61: 851–69.

85. Huemer P, Mutanen M, Sefc KM, Hebert PDN. Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic distances have small genetic impacts. PLOS ONE. 2014;9: e115774.

86. Ashfaq M, Akhtar S, Rafi MA, Mansoor S, Hebert PDN. Mapping global biodiversity connections with DNA barcodes: Lepidoptera of Pakistan. PLOS ONE. 2017;12: e0174749.

87. Ashfaq M, Blagoev G, Tahir HM, Khan AM, Mukhtar MK, Akhtar S, et al. Assembling a DNA barcode reference library for the spiders (Arachnida: Araneae) of Pakistan. PLOS ONE. 2019;14: e0217086.

88. Irwin ME, Kampmeier GE, Weisser WW. Aphid movement: process and consequences. In: Emden HF, van Harrington R, editors. Aphids as crop pests. 2007; p. 153–186.

514 89. Harrington R, Verrier P, Denholm C, Hulle M, Maurice D, Bell N, et al. EXAMINE
515 (EXploitation of Aphid Monitoring in Europe): an European thematic network for the
516 study of global change impacts on aphids. In: Simon JC, Dedryver CA, Rispe C, Hulle
517 M, editors. Aphids in a New Millennium. Science Update 2004. p. 45–49.
518 90. Hull M, Renoust M, Turpeau E. New aphid species detected by permanent aerial
519 sampling programmes in France. In: Nieto Nafria JM, Dixon AEG, editors. Aphids in
520 natural and managed ecosystems. Universidad de Leon, Leon, Spain. 1998. pp. 365–369.
521 91. Miller GL, Halbert SE, Footit RG. North American adventive aphids: appraising the
522 faunal components. 7th International Symposium on Aphids, Freemantle Australia. 2005.
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525 Figure legends:

526 Figure 1. Collection sites for aphids in Pakistan. The map was generated by
527 www.simplemappr.net using GPS coordinates.

528 Figure 2. Barcode gap analysis for species of aphids with three or more specimens collected in
529 Pakistan. NN = nearest neighbor.

530 Figure 3. NJ analysis of COI-5′ sequences from species/ BINs of aphids from Pakistan.
531 Bootstrap values (%) (1,000 replicates) are shown above the branches (values <50% are not
532 shown) while the scale bar shows K2P distances. The node for each species/BIN with multiple
533 specimens was collapsed to a vertical line or triangle, with the horizontal depth indicating the
534 level of intraspecific divergence. BIN numbers are shown for species with only family- or genus-
535 level identification or those split into two BINs.

536 Figure 4**.** Phylogenetic analysis of aphid species/BINs from Pakistan based on COI-5′ sequences.
537 The tree was estimated using Bayesian inference. Posterior probabilities are indicated at nodes.
538 The analysis was based on representative sequences from 67 aphid haplotypes in the dataset that
539 were extracted using DnaSP v5.10 (Librado and Rozas 2009). Taxa are followed by the BINs
540 and haplotype numbers. *Diaphorina citri* (BOLD:AAT8865) was employed as outgroup.

541

542 Supporting Information Legends:

543 S1 Table. Plant-host family range for aphid species/BINs collected in Pakistan.

544 S2 Table. Identification and BIN assignment of 809 specimens of Aphididae collected from 123

545 plant hosts in Pakistan.

546

547 Table 1 Sequence divergence (K2P) in the COI barcode region for aphid species from Pakistan with more than three
548 specimens, genera with three or more species, and families with three or more genera. This analysis only considers
549 specimens that were assigned to a Linnaean species.

Distance class	n	Taxa	Comparisons	Min (%)	Mean (%)	Max (%)
Within Species	764	36	30756	0	0.1	3.6
Within Genus	434	6	32305	0.8	7.4	10.3
Within Family	770	1	233004	3.7	9.6	17.3

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554 Table 2: Geographic (km) and genetic (K2P) distance correlation analysis for 42 aphid species from
555 Pakistan combined with conspecifics from 69 other countries.

Species	Record Count	BINs	Linear Regression R^2	Gen Dist Max	Geo Dist Max	Mantel R^2	Mantel *P* value
Acyrthosiphon malvae	54	2	0.13	4.8	18675	0.13	0.01
Acyrthosiphon pisum	205	1	0.01	1.7	19312	0.01	0.05
Aphis affinis	8	1	0.18	0.2	356	0.18	0.04
Aphis astragalina	37	1	0.71	0.8	10789	0.71	0.01
Aphis craccivora	420	1	0.09	1.4	19417	0.00	0.01
Aphis fabae	426	1	0.01	2.5	19456	0.01	0.01
Aphis gossypii	362	1	0.00	3.9	19369	0.00	0.8
Aphis nasturtii	38	1	0.48	1.6	11656	0.48	0.01
Aphis nerii	99	1	0.02	1.2	19110	0.02	0.01
Aphis spiraecola	277	2	0.00	3.1	19355	0.00	0.2
Aulacorthum solani	118	1	0.13	2.0	19291	0.13	0.01
Baizongia pistaciae	5	2	0.48	4.4	5967	0.48	0.23
Brachycaudus cardui	54	1	0.17	0.9	11929	0.17	0.01
Brachycaudus helichrysi	108	4	0.01	3.1	19426	0.01	0.01
Brevicoryne brassicae	166	2	0.02	6.3	19178	0.02	0.04
Chromaphis juglandicola	11	1	0.52	0.8	10908	0.52	0.01
Hyadaphis coriandri	13	1	0.40	1.4	685	0.40	0.01
Hyalopterus pruni	151	3	0.06	6.6	16867	0.06	0.01
Hyperomyzus lactucae	87	1	0.05	0.3	19474	0.05	0.02
Hysteroneura setariae	53	1	0.04	1.9	15843	0.04	0.01
Lipaphis pseudobrassicae	91	2	0.12	3.6	18212	0.13	0.01
Macrosiphoniella sanborni	5	1	0.01	0.2	15935	0.01	0.43
Macrosiphum euphorbiae	198	1	0.14	1.6	19482	0.14	0.01
Macrosiphum rosae	80	1	0.00	1.2	19379	0.00	0.14
Melanaphis donacis	7	1	0.16	0.2	6092	0.16	0.35
Melanaphis sacchari	225	1	0.02	1.9	18400	0.02	0.01
Myzus persicae	322	1	0.00	2.2	19234	0.00	0.68
Nearctaphis bakeri	70	2	0.04	6.8	11961	0.04	0.09
Periphyllus lyropictus	33	1	0.00	0.2	11002	0.00	0.92
Rhodobium porosum	7	1	0.08	0.2	12350	0.08	0.1
Rhopalosiphum maidis	63	1	0.00	2.0	18405	0.00	0.40
Rhopalosiphum padi	1189	2	0.30	5.0	19841	0.29	0.01
Rhopalosiphum rufiabdominale	18	1	0.03	0.2	15787	0.03	0.99
Sitobion avenae	314	1	0.02	4.6	18405	0.00	0.01
Tetraneura nigriabdominalis	41	2	0.16	8.6	16534	0.16	0.01
Therioaphis trifolii	470	2	0.00	13.0	18823	0.00	0.33
Uroleucon sonchi	45	2	0.11	2.3	19003	0.11	0.03
Acyrthosiphon gossypii	5	1	N/A	N/A	N/A	N/A	N/A
Brachyunguis harmalae	8	1	N/A	N/A	N/A	N/A	N/A
Cinara tujafilina	8	1	N/A	N/A	N/A	N/A	N/A
Sarucallis kahawaluokalani	10	1	N/A	N/A	N/A	N/A	N/A
Schizaphis rotundiventris	5	1	N/A	N/A	N/A	N/A	N/A

556 N/A: Data for the correlation analysis was missing.
557

558 Table 3: Occurrence of 52 pest aphid BINs across six continents and their association with
559 Linnaean species on the Barcode of Life Data System (BOLD).

BIN	Countries	Continents	(Number) and names of the associated species
AAA3070	44	6	(35) *Aphis affinis, A. aliena, A. argrimoniae, A. cf. frangulae, A. chloris, A. cisticola, A. clerodendri, A. confusa, A. crepidis, A. egomae, A. frangulae, A. gossypii, A. hieracii, A. hypericiphaga, A. hypochoeridis, A. idaei, A. leontodontis, A. lichtensteini, A. longirostrata, A. madderae, A. mamonthovae, A. monardae, A. nivalis, A. oestlundi, A. origani, A. parietariae, A. punicae, A. ruborum, A. sedi, A. serpylli, A. sumire, A. taraxacicola, A. teucrii, A. viticis*
AAA3759	15	6	(1) *Therioaphis trifolii*
AAA4183	29	6	(1) *Aphis spiraecola*
AAA5565	31	6	(9) *Aphis fabae, A. solanella, A. hederae, A. ilicis, A. viburni, A. newtoni, A. fukii, A. lambersi, A. seselii*
AAA6213	19	6	(13) *Macrosiphum albifrons, M. cerinthiacum, M. cholodkovskyi, M. corydalis, M. daphnidis, M. euphorbiae, M. gaurae, M. gei, M. hellebori, M. impatientis, M. sileneum, M. valerianae, M. zionense*
AAA7683	22	6	(1) *Myzus persicae*
AAA9899	16	4	(1) *Rhopalosiphum padi*
AAB1787	19	6	(1) *Acyrthosiphon pisum*
AAB2572	16	5	(2) *Aulacorthum solani, Macrosiphum gei*
AAB4239	20	4	(3) *Macrosiphum rosae, M. funestum, Sitobion rosivorum*
AAB4894	17	5	(1) *Sitobion avenae*
AAB6874	10	5	(6) *Ericaphis scammelli, E. fimbriata, Rhodobium porosum, Wahlgreniella nervata, W. vaccinii, W. arbuti*
AAB7937	30	6	(8) *Aphis craccivora, A. masoni, A. intybi, A. rumicis, A. spiraecola, A. tirucallis, A. coronillae, A. fabae*
AAB8566	20	5	(2) *Hyperomyzus lactucae, H. carduellinus*
AAB9726	14	6	*(1) Brachycaudus helichrysi*
AAC1165	13	5	*(2) Brachycaudus cardui, B. lateralis*
AAC1372	22	6	(1) *Aphis nerii*
AAC1374	8	3	(5) *Aphis nasturtii, A. davletshinae, A. umbrella, A. althaeae, A. cf. rostella*
AAD0145	18	6	(1) *Brevicoryne brassicae*
AAD0902	4	3	(1) *Nearctaphis bakeri*
AAD4538	12	6	(1) *Rhopalosiphum maidis*
AAD9153	11	6	(2) *Lipaphis pseudobrassicae, L. erysimi*
AAE2497	13	5	(1) *Hysteroneura setariae*
AAG3896	14	5	(1) *Tetraneura nigriabdominalis*
AAG6658	6	3	(1) *Chromaphis juglandicola*
AAH2863	4	3	(1) *Periphyllus lyropictus*
AAI0406	13	5	(1) *Rhopalosiphum rufiabdominale*
AAI4332	3	2	(NA) Identified to genus – *Aphis*
AAI7650	2	1	(1*) Baizongia pistaciae*
AAK5331	3	2	(1) *Hyadaphis coriandri*
AAK7235	22	5	(2) *Melanaphis sacchari, M. japonica*

AAM0964	7	4	(2) *Macrosiphoniella yomogifoliae, M. abrotani*
AAN2425	5	4	(1) *Macrosiphoniella sanborni*
AAN4898	2	2	(1) *Brachyunguis harmalae*
AAO7083	6	3	(1) *Cinara tujafilina*
AAP9276	5	3	(NA) Identified to genus – *Schizaphis*
AAX9332	4	3	(1) *Sarucallis kahawaluokalani*
AAY6004	4	2	(1) *Melanaphis donacis*
ACD8115	2	1	(1) *Hyalopterus pruni*
ACF2924	11	6	(1) *Rhopalosiphum padi*
ACI9922	5	3	(NA) Identified to genus – *Capitophorus*
ACO4203	4	2	(1) *Schizaphis rotundiventris*
ACO5373	2	1	(1) *Hyalopterus pruni*
ACS1400	2	1	(1) *Acyrthosiphon gossypii*
ABY0239	1	1	(NA) Identified to genus – *Aphis*
ACP3887	1	1	(NA) Identified to genus – *Forda*
ACS1208	1	1	(NA) Identified to genus – *Acyrthosiphon*
ACS1445	1	1	(1) *Acyrthosiphon malvae*
ACS2175	1	1	(NA) Identified to subfamily – *Aphidinae*
ACT3010	1	1	(NA) Identified to subfamily – *Aphidinae*
ACV1458	1	1	(1) *Uroleucon sonchi*
ACV6041	1	1	(NA) Identified to genus – *Aphis*

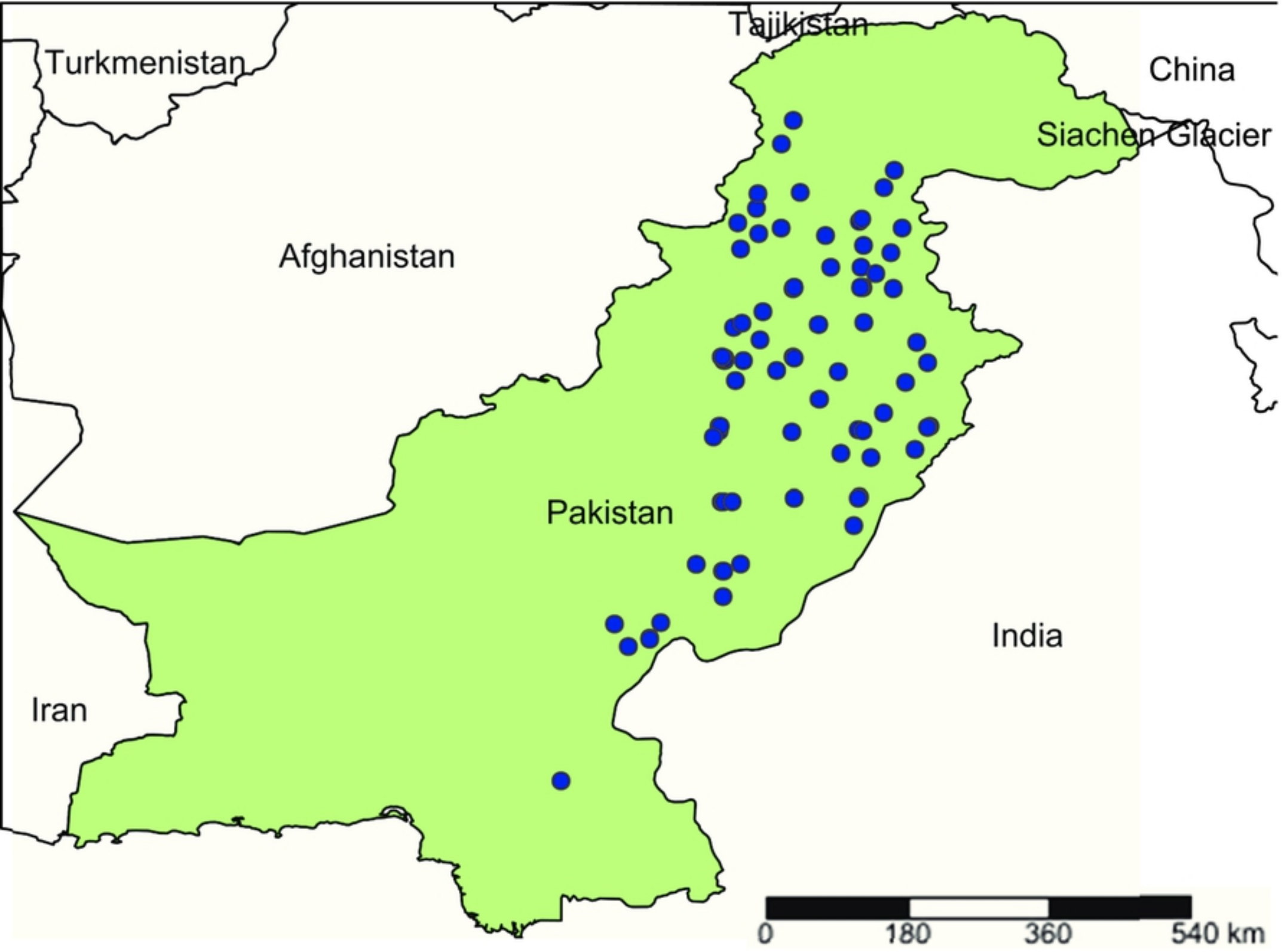
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Figure 1

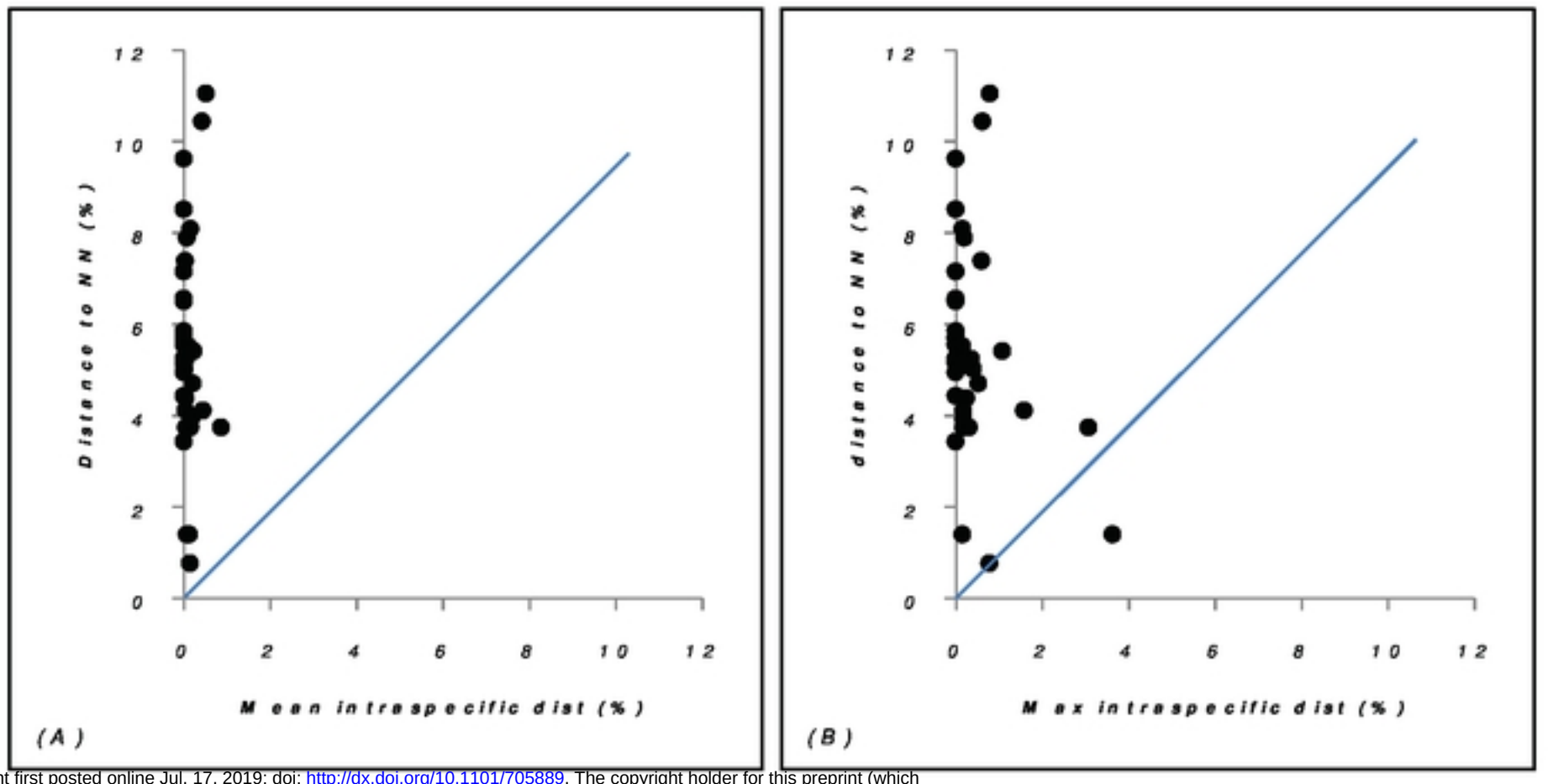

Figure 2

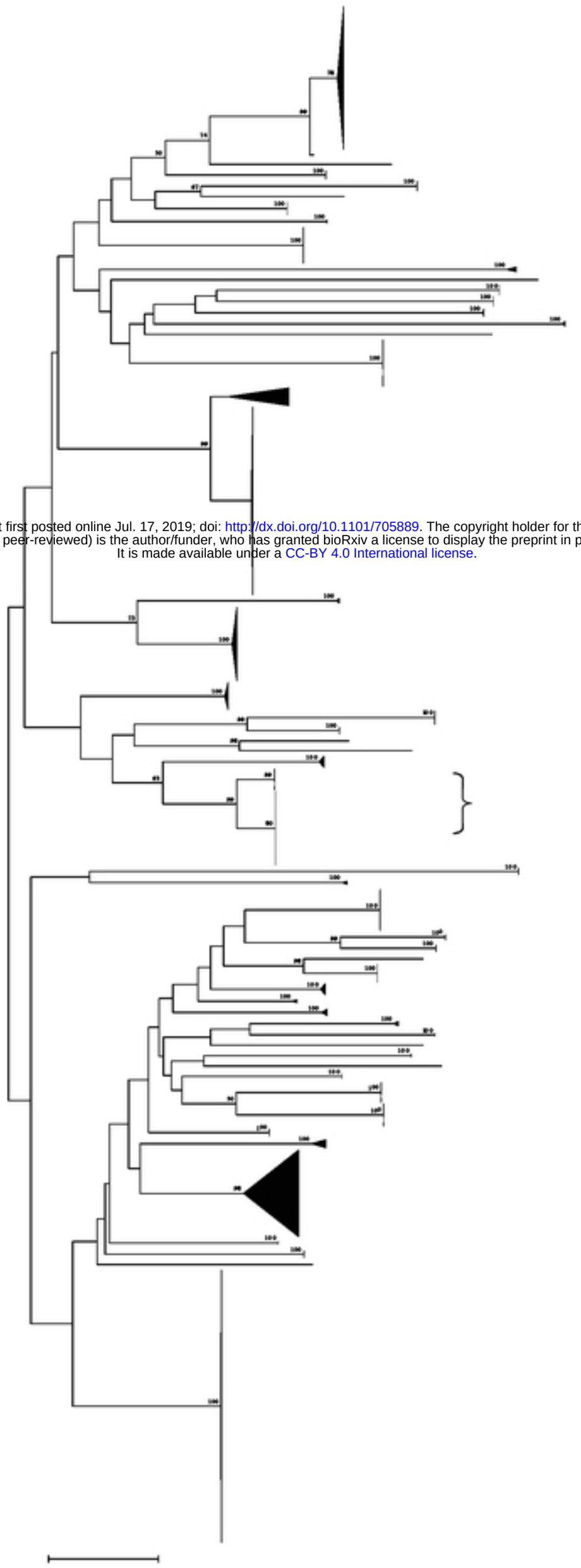

Figure 3

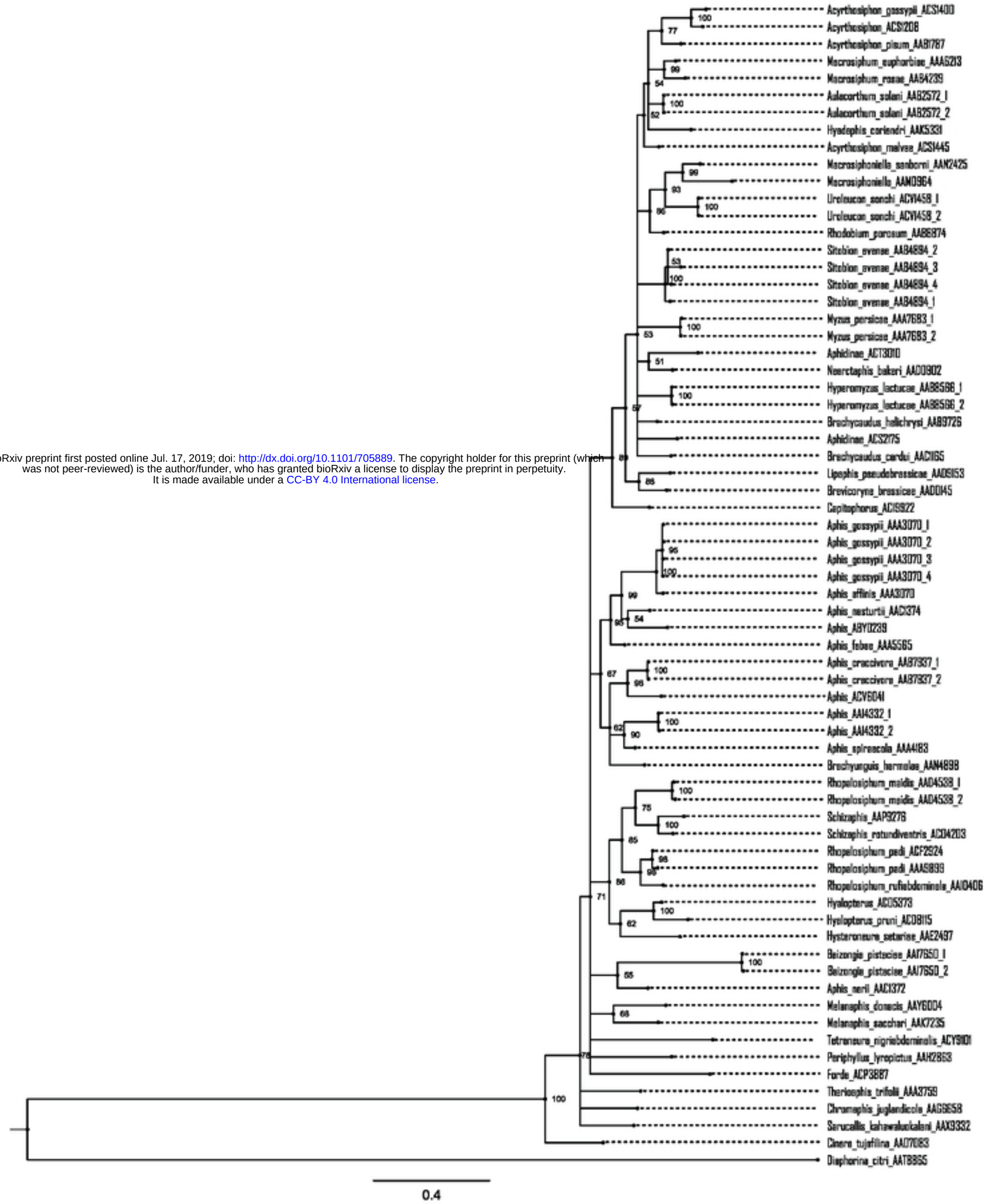

Figure 4