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# Comparative Phylogeny of the Genus *Bordetella* Using Sequence Analysis of 16S rRNA and *ompA* Genes

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ARTICLE INFO	ABSTRACT
Article type: Original Article	<b>Background</b> : The genus Bordetella harbors 16 species; three of them are well-known for their high medical importance. The phylogenetic diversity of the genus is currently not very well investigated.
Article history: Received: 19 Jan 2017 Revised: Jun Mar 2017	<i>Methods</i> : In this study, 16S rRNA gene sequence of 16 type strains of the <i>Bordetella</i> species were analyzed. Also, phylogenies conducted on the same gene of 247 isolates of <i>Bordetella</i> species, comprising a wide physiological as well as ecological diversity and encompassing ex-type representatives of the 16 <i>Bordetella</i> species, were analyzed.
Accepted: 11 Sep 2017 Published: 15 Oct 2017	<b>Results:</b> It was found that the phylogenetic diversity of the genus may be very different from that is currently assumed. Interestingly, the 16S rRNA gene signals could not resolve some species with
Keyworas: Alcaligenaceae,	promising bootstrap and posterior probability values as our phylogenies, using maximum likelihood and Bayesian inference methods, showed.
Biogeography, Bordetella species, Ecological distribution, Phylogenetic species concept.	<b>Conclusion:</b> Our results indicate a probable need for additional phylogenetic signals which can be provided by coding genes. Therefore, sequence data of <i>ompA</i> gene of <i>Bordetella</i> species, a critically significant genomic region in pathogenesis, was here analyzed, phylogenetically. This gene confirmed the tree topology and the phylogenetic species boundaries already revealed by the 16S rRNA gene, but showed a better discriminatory power which resolved <i>Bordetella</i> species with higher statistically significant values.

*Please cite this paper as:* Badamchi A, Papizadeh M. Comparative Phylogeny of the Genus *Bordetella* Using Sequence Analysis of 16S rRNA and *ompA* Genes. *J Med Bacteriol*. 2017; **6** (3, 4): pp.1-13.

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#### Introduction

There is a considerable number of opportunistic bacterial pathogens in various environmental samples including soils and feces (1, 2). Various soils are known as the origin of various nonpathogenic and either pathogenic microbial species. Thus, various species of opportunistic bacterial pathogens, likely *Enterobacteriaceae* (3), *Microbacterium* (4), *Pseudomonas* (5), *Stenotrophomonas* (6), and many other genera of the kingdom Eubacteria can be detected in various soils, abundantly.

The genus *Bordetella* includes 16 well-known species among which three species: *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*, have a very higher biomedical importance (7, 8). According to the previously published reports, *Bordetella* species have been mainly found as pathogens, but also various environmental samples; soil, water, and air are regarded as their habitats (7, 8). Recent findings suggest soil as a probable environmental origin of *Bordetella* species, including the animalpathogenic lineages (7-9). The significant abundance of pathogenic *Bordetella* species in soils explains their wide distribution as well as frequent disease outbreaks that start without an obvious infectious source (9, 10).

B. pertussis is a strict human pathogen causing the respiratory tract infection called whooping cough (9). B. parapertussis consists of two lineages, one infecting human and the other infecting sheep (10). In contrast to these single host adapted pathogens, B. bronchiseptica: a close species to the two above species can cause a broad array of respiratory diseases (11, 12). B. trematum is a nonpathogenic, opportunistic organism whose sole source of isolation is thought to be open wounds of humans (13). In place, B. trematum causes ear and wound infections (14). A selective microbe-host association between B. trematum and B. holmesii species, and humans seems probable since these two Bordetella species have been exclusively detected as human pathogens (14). B. holmesii has been found repeatedly in blood and often in sputum of adolescents and is an emerging

cause of septic arthritis (15-17). B. avium, a pathogen of birds, causes coryza or rhinotracheitis in poultry, but it has never been found in humans. B. petrii, causes sinusitis in immunocompromised adolescents, has been isolated from environmental samples and is capable of anaerobic growth (8, 18). B. hinzii, mainly colonizes the respiratory tract of poultry, has been also found as a chronic cholangitis infection agent in immunocompromised humans (19) and was recently reported as a causative agent of fatal septicemia (20). Since B. hinzii has been isolated from trachea and lungs of laboratory mice with respiratory infection and wild rodents, it is assumed that these animals may serve as reservoir for this species that could be transmitted to human or pets (19, 20). B. hinzii should be added to the list of emerging bacterial zoonotic agents in wild rodents that could be pathogenic for humans, especially immunocompromised patients (20, 21). B. pseudohinzii; a close species to B. hinzii, is also detected as a rodent-associated *Bordetella* species (19-22). B. bronchialis, B. flabilis, and B. sputigena (23, 24) are recently introduced and they have been isolated from human respiratory specimens. In contrast to other bordetellae, B. trematum (21) and B. ansorpii (22) are not associated with respiratory problems but are isolated from human wound infections.

Species delimitation seems to be difficult dealing with bordetellae. *B. hinzii* is highly difficult to become differentiated from *B. pseudohinzii* and even *B. avium* by routine phenotypic methods. Similarly, miss-identification is highly probable differentiating *B. parapertussis*, *B. pertussis* and *B. bronchiseptica* (24, 25). Sequence-based identification and phylogeny tend to be a promising approach to resolve the species boundaries (26).

Considering the increasing rate of the researches performed on various bacterial species to fulfill sequence-based identifications, the phylogenetic species boundaries have become faint. Thus, a single genomic locus may become exhausted from the needed signals to resolve very close species Confirmation can be reliably achieved using

advanced genotypic and phylogenetic methods (24, 27), and the greater nucleotide variation of the conserved protein coding genes allows unequivocal identification of very close *Bordetella* species. Thus, in this study we performed a preliminary research on the applicability of *OmpA* gene sequence, encoding a porin-like protein which has a critical role in pathogenesis, in phylogeny and identification of *Bordetella* species.

#### **Materials and Methods**

The 16S rRNA gene reference sequences and either the same gene sequences from isolates and uncultured materials obtained from Ribosomal Database Project (27, 28). Also, *ompA* gene sequences obtained from the nucleotide database of GenBank, NCBI. Thus, three different datasets (two datasets for 16S rRNA gene and a dataset for the nucleotide sequence of the coding gene for *ompA*) were prepared, separately.

The datasets were aligned with the multiple sequence alignment tool; Multiple sequence Alignment using Fast Fourier Transform (MAFFT), available European at the Bioinformatics Institute (EMBL-EBI), separately (29-35). Alignments were manually improved in MEGA v. 7.0.9 and Bioedit v. 7.0.5.3 packages (default settings) (36, 37). Maximum likelihood and Bayesian analyses were conducted using separated or concatenated datasets. The online tool Findmodel (http://www.hiv.lanl.gov/content/ sequence/findmodel/ findmodel.html) was used to determine the best nucleotide substitution model for each partition. Bayesian inference (BI) analysis was conducted for each dataset, separately. Bayesian analyses were conducted with MrBayes v3.2.1 (38) executed on XSEDE (Extreme Science and Engineering Discovery Environment) through the CIPRES Science Gateway v. 3.3 (39) in two parallel runs, using the default settings but with the following modifications: general time reversible

(GTR) model of DNA substitution as the best fit and a gamma distribution rate variation across sites (29). This model was chosen as the result from a pretest with MrModeltest v. 2.2 (40). After this was determined, the GTR + I + G model, as the best nucleotide substitution model, was used for the combined dataset, and a MCMC heated chain was set with a temperature value of 0.05. The number of chains, number of generations, and sample frequencies were set, respectively, at 4, 20000000 or 5000000, and 1000. Chain convergence was determined using Tracer v. 1.5 (http://tree.bio.ed.ac.uk/ software/tracer/) to confirm sufficiently large ESS values (>200). The sampled trees were subsequently summarized after omitting the first 25 % of trees as burn-in using the "sump" and "sumt" commands implemented in MrBayes (41, 42). The tree was visualized and edited using FigTree v. 1.4.2 (43, 32).

#### Results

Sequence dataset of the 16S rRNA gene which was provided by RDP database contained sequences of type strains, isolates and uncultured sequence data. The sequence data of this gene was screened and split into three separate alignments; type strains, isolates and uncultured sequence. Besides, sequence dataset of ompA gene was produced using the similarity search engines of BLAST program available at NCBI. The tree topology for the three separate alignments of the 16S rRNA gene was the same. Thus, the 16S rRNA gene alignments of the type strains and isolates were fused and used for further analyses (Fig. 1). Also, to infer the familial placement of the genus Bordetella, 16S rRNA gene sequences for the type strains of the genus Bordetella were analyzed in an which contained alignment the reference sequences for the genera of Alcaligenaceae and allied families.

	Bordetella bronchiseptica X57026
	- Bordetella parapertussis BX640428
	Bordetella parapertussis BX640434
	Bordetella bronchiseptica BX640442
	Bordetella bronchiseptica BX640447
	Bordetella bronchiseptica BX640449
	Bordetella bronchiseptica E965806
	Bordetella bronchiseptica HE965806
	Bordetella bronchiseptica HE965807
	Bordetella bronchiseptica HE965807
	Bordetella bronchiseptica HE965807
91	Bordetella bronchiseptica HE965807
	Bordetella pertussis BX640417
	Bordetella pertussis BX640418
	Bordetella pertussis BA040420 Rordetella pertussis HE965805
	Bordetella pertussis HE965805
	Bordetella pertussis CP009751
	Bordetella pertussis CP009751
	Bordetella pertussis CP009751
	Bordetella pertussis CP009752
	Bordetella pertussis CP009752
	Bordetella pertussis CP010323
	Bordeteila pertussis CP010323
	Bordetella pertussis CP010323
	Bordetella pertussis CP011400
	- Rordetella pertussis CP011400
	Bordetella pertussis CP011400
	Bordetella pertussis CP011401
	Bordetella pertussis CP011401
	Bordetella pertussis CP011440
	Bordetella pertussis CP011440 Bordetella pertussis CP011440
	- Bordetella pertussis CP011441
	Bordetella pertussis CP011441
	Bordetella pertussis CP011441
	Bordetella pertussis CP011442
	Bordetella pertussis CP011442
	Bordetella pertussis CP011442
	Borderella pertussis CP011443
	Bordetella pertussis CP011443
	Bordetella pertussis CP011444
	100 Bordetella pertussis CP011444
	Bordeteila pertussis CP011444
	Bordetella pertussis CP011445
	Bordetella pertussis CP011445
	Bordetella pertussis CP011446
	Bordetella pertussis CP011446
	Bordetella pertussis CP011446
	Bordetella pertussis CP011447
	Bordetella pertussis CP011447
	Bordeteila pertussis CP011448
	Bordetella pertussis CP011448
	Bandata IIa anatomia CDO11440
	Bordetena pertussis CP011448
	Bordetella pertussis CP011448 Bordetella pertussis BX470248
	Bordetella pertussis CPU11446     Bordetella pertussis BVA70248     Bordetella pertussis CP002695     Bordetella pertussic CP002695
	Aordeteila pertusis SC 1011446     Bordeteila pertusis SC 1002695     Bordeteila pertusis SC 1002695     Bordeteila pertusis C 1002695     Bordeteila pertusis C 1002695
	Aonderlin pertussis CF011446     Aonderlin pertussis CF001248     Bonderlin pertussis CF002295     Bonderlin pertussis CF002295     Bonderlin pertussis CF002295     Fonderlin pertussis CF002295     Fonderlin pertussis SAX10248
	Honderbin pertussis CP011446     Honderbin pertussis CP01248     Bonderbin pertussis CP002695     Bonderbin pertussis CP002695     Bonderbin pertussis CP002695     Bonderbin pertussis BX470248     Bonderbin pertussis BX470248
	Aordetella pertussis CP011446     Fordetella pertussis CP02695     Bordetella pertussis CP02695     Bordetella pertussis CP02695     Fordetella pertussis CP02695     Fordetella pertussis CP02695     Bordetella pertussis RX470248     Bordetella pertussis HE965805     Bordetella pertussis HE965805
	Aonderia permissis CP01144a     Aonderia permissis CP01244a     Bonderia permissis CP002695     Bonderia permissis CP002695     Bonderia permissis ASV10248     Bonderia permissis BX470248     Bonderia permissis BX470248     Bonderia permissis BX470248     Bonderia permissis H1905805     Bonderia permissis H1905805
	Honderbal pertussis VE1011446     Honderbal pertussis VE1011446     Honderbal pertussis VE1002695     Honderbal pertussis CP002695     Honderbal pertussis VE101248     Bonderbal pertussis BX470248     Bonderbal pertussis BX470248     Honderbal pertussis H1965805     Honderbal pertussis H1965805     Honderbal pertussis H1965805     Gonderbal pertussis H1965805     Honderbal pertussis H1965805
	Bonderlah permissi K-P011448     Fonderlah permissi K-P012498     Bonderlah permissi K-P0022995     Bonderlah permissi K-P0022995     Honderlah permissi K-P0022995     Honderlah permissi K-P002498     Bonderlah permissi K-P00248     Bonderlah permissi K-P005405     Honderlah permissi K-P005405     Honderlah permissi K-P005494     Bonderlah holmissi C-P007494     Bonderlah holmissi C-P007494
	Aordeela pertussis CP01144a     Aordeela pertussis CP01244a     Bordeela pertussis CP002695     Bordeela pertussis CP002695     Bordeela pertussis ASX10248     Bordeela pertussis BX470248     Bordeela pertussis BX470248     Bordeela pertussis H9058005     Bordeela pertussis H9058005     Bordeela pertussis H9058005     Bordeela pertussis H9058005     Bordeela pertussis (P907494     Bordeela holmesii CP007494     Bordeela holmesii CP007494     Bordeela holmesii CP007494
	Horderlah pertussis VE/011446     Horderlah pertussis VE/002695     Horderlah pertussis VE/002695     Horderlah pertussis VE/002695     Horderlah pertussis VE/002695     Horderlah pertussis VE/02488     Borderlah pertussis VE/02488     Horderlah pertussis VE/
	Bonderella pertussis VEPUT448     Bonderella holmesii CPUT494     Bonderella holmesii KB683187     Bonderella holmesii AV466115
	Aordeela periosis VEV01446     Aordeela periosis VEV01446     Bordeela periosis VEV02695     Bordeela periosis CP002695     Bordeela periosis VEV02695     Bordeela periosis VEV02695     Bordeela periosis VEV0248     Bordeela periosis VEV05805     Bordeela periosis VEV05805     Bordeela periosis VEV05805     Bordeela periosis VEV0544     Bordeela holmesii CP007494     Bordeela holmesii CP007494     Bordeela holmesii AE66579     Bordeela holmesii AE
	Honderlin pertussis VF011446     Honderlin pertussis VF002695     Honderlin pertussis VF0026805     Honderlin pertussis VF005805     Honderlin pertussis VF005805     Honderlin pertussis VF005805     Honderlin pertussis VF007494     Honderlin holmesii CP007494     Honderlin holmesii AP466115     Honderlin holmesii AP466115     Honderlin holmesii AP466116     Honderlin holmesii AP466114
	Aonderlah pertussis VEP011445     Aonderlah pertussis VEP01248     Bonderelih pertussis VEP012695     Bonderelih pertussis CP002695     Bonderelih pertussis VEP01248     Bonderelih pertussis VEP01248     Bonderelih pertussis VEP05805     Bonderelih pertussis VEP05805     Bonderelih holmesii CP007494     Bonderelih holmesii CP007494     Bonderelih holmesii R406519     Bonderelih holmesii R406519     Bonderelih holmesii AV466115     Bonderelih holmesii AV466115     Bonderelih holmesii AV466114     Bonderelih holmesii AV
	Aordeetia pertussis Verbi 144a     Aordeetia pertussis Verbi 244a     Dordeetia pertussis Verbi 244a     Dordeetia pertussis CP002695     Bordeetia pertussis 048470248     Dordeetia pertussis 048470248     Dordeetia pertussis 048470248     Dordeetia pertussis 04870248     Dordeetia pertussis 04870505     Dordeetia pertussis 0480505     Dordeetia pertussis 048051     Dordeetia pertussis 04905494     Tordeetia holmesii CP007494     Tordeetia holmesii CP007494     Tordeetia holmesii A166519     Dordeetia holmesii A166519     Dordeetia holmesii A1665116     Tordeetia holmesii X1290044     Dordeetia holmesii X129044     Dordeetia holmesi X12904     Dordeetia holmesi X12904
	Aonderbia perinsis SC P011445     Aonderbia perinsis SC P012995     Bonderbia perinsis SC P002995     Bonderbia perinsis SC P002995     Honderbia perinsis SC P002995     Honderbia perinsis SK P00248     Donderbia perinsis SK P10248     Donderbia perinsis SK P10248     Donderbia perinsis SK P1055005     Honderbia perinsis SK P1055005     Bonderbia perinsis SK P1055005     Bonderbia holmesii CP007494     Bonderbia holmesii R456519     Bonderbia holmesii R4566115     Bonderbia holmesii R4566114     Bonderbia holmesii R4566114     Bonderbia holmesii R4566114     Bonderbia holmesii R456529
	Aondreini perinsis Verbi 1445     Aondreini perinsis Verbi 1445     Bondreini perinsis Verbi2995     Bondreini perinsis CP002095     Bondreini perinsis Verbi295     Bondreini perinsis Verbi29580     Bondreini perinsis Verbi295805     Bondreini perinsis Verbi295     Bondreini Nolmesii AP366579     Bondreini Nolmesii AP366579     Bondreini Nolmesii AP366115     Bondreini Nolmesii AP366115     Bondreini Nolmesii AP366116     Bondreini Nolmesii AP360116     Bondreini Nolmesii AP360116     Bondreini Nolmesii AP360116     Bondreini Nolmesii X1239044     Bondreini holmesii X239044     Bondreini holmesii X239044     Bondreini holmesii X239044     Bondreini holmesii X239044     Bondreini Molmesii X239044     Bondreini Molmesii X239044     Bondreini Molmesii M239044     Bondreini M239
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	Aonderbia perinsis SC P011445     Aonderbia perinsis SC P012695     Bonderbia perinsis SC P002695     Bonderbia perinsis SC P002695     Honderbia perinsis SC P002695     Honderbia perinsis SK P002488     Bonderbia perinsis SK P002488     Bonderbia perinsis SK P102488     Bonderbia holmesii CP007494     Bonderbia holmesii CP007494     Bonderbia holmesii CP007494     Bonderbia holmesii AP366579     Bonderbia holmesii AP366515     Bonderbia holmesii AP366115     Bonderbia holmesii AP3046114     Bonderbia holmesii AP30490126     Bonderbia holmesii AP3049012     Bonderbia holmesii AP365529     Fonderbia holmesii AP365529     Fonderbia holmesii AP365529     Fonderbia holmesii AP365529     Fonderbia holmesii CP007495     Fonderbia holmesii CP00
	Aonderbin perinsis K-P01144a     Aonderbin perinsis K-P01248     Bonderbin perinsis K-P002695     Bonderbin perinsis K-P002695     Bonderbin perinsis K-P002695     Bonderbin perinsis K-P02488     Bonderbin perinsis K-P05805     Bonderbin holmesii C-P077494     Bonderbin holmesii AF466116     Bonderbin holmesii AF4566116     Bonderb
	<ul> <li>Douberbil pertussis VEP011446</li> <li>- Ronderellin pertussis VEP012995</li> <li>- Bonderellin pertussis CP002995</li> <li>- Bonderellin pertussis CP002995</li> <li>- Bonderellin pertussis VEP05805</li> <li>- Bonderellin holmessii CP007494</li> <li>- Bonderellin holmessii CP007495</li> <li>- Bonderellin holmessii DQ107495</li> <li>- Bonderellin holmessii CP007495</li> <li>- Bonderellin holmessii CP007495</li> <li>- Bonderellin holmessii CP007495</li> <li>- Bonderellin holmessii CP007495</li> <li>- Bonderellin holmessii VQ40435</li> <li>- Bonderellin holmessii VQ413232</li> </ul>
	Aonderbin perinsis K-P01144a     Aonderbin perinsis K-P01248     Bonderbin perinsis K-P012695     Bonderbin perinsis K-P012695     Bonderbin perinsis K-P01268     Bonderbin perinsis K-P01248     Bonderbin perinsis K-P01248     Bonderbin perinsis K-P0248     Bonderbin perinsis K-P02484     Bonderbin perinsis K-P02484     Bonderbin perinsis K-P02494     Bonderbin holmesii CP007494     Bonderbin holmesii K-P02494     Bonderbin holmesii K-P02494     Bonderbin holmesii AP366579     Bonderbin holmesii AP366115     Bonderbin holmesii AP466115     Bonderbin holmesii AP466114     Bonderbin holmesii AP366114     Bonderbin holmesii AP366114     Bonderbin holmesii AP366115     Bonderbin holmesii AP369012     Bonderbin holmesii AP3690136     Bonderbin holmesii CP0071495     Bonderbin holmesii KF601905     Bonderbin holmesii KF601905
	<ul> <li>Douberbil pertussis VE/011445</li> <li>Honderellin pertussis VE/002695</li> <li>Bonderellin pertussis CP002695</li> <li>Bonderellin pertussis SAV0248</li> <li>Bonderellin pertussis SAV0248</li> <li>Bonderellin pertussis SAV0248</li> <li>Bonderellin pertussis SAV0248</li> <li>Bonderellin pertussis BAV0248</li> <li>Bonderellin pertussis HP058005</li> <li>Bonderellin pertussis (PD07494</li> <li>Bonderellin holmessii CP007494</li> <li>Bonderellin holmessii (P007494</li> <li>Bonderellin holmessii AF366579</li> <li>Bonderellin holmessii AF400444</li> <li>Bonderellin holmessii AF406115</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF356239</li> <li>Bonderellin holmessii AF366579</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF366576</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF35623</li> <li>Bonderellin holmessii AF466116</li> <li>Bonderellin holmessii AF4860125</li> <li>Bonderellin holmessii AF4860126</li> <li>Bonderellin holmessii AF4860126</li> <li>Bonderellin holmessii AF486114</li> <li>Bonderellin holmessii AF480002</li> <li>Bonderellin holmessii AF48002</li> <li>Bonderellin holmessii A</li></ul>
	Aonderlah perinsis K-P011445     Aonderlah perinsis K-P01248     Bonderlah perinsis K-P012695     Bonderlah perinsis K-P012695     Honderlah perinsis K-P012695     Honderlah perinsis K-P01248     Bonderlah holmesii CP007494     Bonderlah holmesii K-P01244     Bonderlah holmesii K-P01245     Bonderlah holmesii K-
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	Bordetella parapertussis AF366578
	100_Bordetella parapertussis HE965803
	Bordetella parapertussis AF366577
	Bordetella bronchiseptica AB680479 Bordetella bronchiseptica AJ278452
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	-Bordetella sp EU082141
	<i>Bordetella</i> sp. EU082150 <i>Bordetella</i> sp. EU082145
	Bordetella sp. EU082142 Bordetella sp. EU082163
	-Bordeteilla sp EU082137
	Bordetella sp EU082152 Bordetella sp EU082153
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9	Bordetella bronchiseptica 10933663           Bordetella bronchiseptica 1093363           Bordetella bronchiseptica 1093363     <
9	Bordetella binacii CP012076       Bordetella binacii CP012077       Bordetella binacii NATA\$269       Bordetella binacii NATA\$2757       Bordetella binacii XP801904       Bordetella binacii XP801904       Bordetella binacii XP8019059       Bordetella binacii XP801725
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9	Bordetella bronchiseptica 1993663 Bordetella bronchiseptica 1993663 Bordetella bronchiseptica 1993663 Bordetella bronchiseptica 1993663 Bordetella bronchiseptica 1993663 Bordetella hinaii CP012076 Bordetella hinaii CP012076 Bordetella hinaii CP012077 Bordetella hinaii U082161 Bordetella hinaii EU082163 Bordetella hinaii EU082164 Bordetella hinaii EU082165 Bordetella hinaii EU082175 Bordetella hinaii EU082175 Bordetella hinaii EU082175 Bordetella hinaii EU082176 Bordetella hinaiii EU082176 Bordetella hi
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**Figure 1.** The Bayesian inference phylogeny of the members of the genus *Bordetella* based on the 16S rRNA gene sequence data. Bayesian posterior probabilities above 0.75 resulting from 50,000,000 replicates are given at the nodes. The GenBank accessions are given after the species names. Species are differentiated with alternative colours (putative undescribed species are not highlighted). Type strains of the described *Bordetella* species are shown in bold. The tree is rooted to *Alcaligenes fecalis*.



**Figure 2.** The Bayesian inference phylogeny of the genus *Bordetella* based on the 16S rRNA gene sequence data of type strains. Bayesian posterior probabilities above 0.75 resulting from 20,000,000 replicates are given at the nodes. The GenBank accessions are given after the species names. The genera *Bordetella* and *Achromobacter* are differentiated with alternative colours. The tree is rooted to *Derxia gummosa*.

Bordetella pertussis strain H788 Bordetella pertussis strain I498 Bordetella pertussis strain 1669 Bordetella pertussis strain 1707 Bordetella pertussis strain H800 Bordetella pertussis strain H812 Bordetella pertussis strain I228 Bordetella pertussis strain 1373 Bordetella pertussis strain 1752 Bordetella pertussis strain H320 Bordetella pertussis strain H834 Bordetella pertussis strain H710 Bordetella pertussis strain H851 Bordetella pertussis strain H915 Bordetella pertussis strain 1975 Bordetella pertussis strain J022 Bordetella pertussis strain 1344 Bordetella pertussis strain C505 Bordetella pertussis strain C742 Bordetella pertussis strain D175 Bordetella pertussis strain D321 Bordetella pertussis strain H346 Bordetella pertussis strain H437 Bordetella pertussis strain H775 Bordetella pertussis strain H779 Bordetella pertussis strain H787 Bordetella pertussis strain H864 Bordetella pertussis strain I110 Bordetella pertussis strain I127 Bordetella pertussis strain I136 Bordetella pertussis strain I331 Bordetella pertussis strain I375 Bordetella pertussis strain I380 Bordetella pertussis strain I386 Bordetella pertussis strain I728 Bordetella pertussis strain 1755 Bordetella pertussis strain 1959 Bordetella pertussis strain 1965 Bordetella pertussis strain 1968 Bordetella pertussis strain J023 Bordetella pertussis strain J076 Bordetella pertussis strain H681 Bordetella pertussis strain 1751

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0.02

**Figure 3.** The Bayesian inference phylogeny of the members of the genus *Bordetella* based on the sequence data of the coding gene for *ompA*. Bayesian posterior probabilities above 0.75 resulting from 20,000,000 replicates are given at the nodes. The GenBank accessions are given after the species names. Species are differentiated with alternative colours. The tree is rooted to *Achromobacter insolitus* (DSM 23807).

 Table 1.
 Genomics data of some Bordetella species available in the genome database of GenBank, NCBI.

	Genome	Median	Median	Median GC
	assemblies	total length	protein count	content (%)
		(Mb)	1	
B. bronchiseptica	68	5.19	4759	68.2
B. parapertussis	4	4.78	4162	68.1
B. pertussis	613	4.05	3576	67.7
B. holmesii	21	3.61	3139	62.7
B. hinzii	10	4.89	4456	67
B. petrii	3	5.04	4718	65.5
B. avium	2	3.71	3262	61.6
B. pseudohinzii	4	4.53	4124	66.6
B. trematum	5	4.44	3985	65.7
B. ansorpii	2	6.17	5357	66.8
B. flabilis	2	5.95	5238	65.9
B. bronchialis	2	5.92	5144	67.3

an intraspecies diversity can also be observed in *B. petrii* clade (Fig. 1).

Phylogenies performed on the coding gene for *ompA* confirmed the efficient variability of the nucleotide sequence of this gene which resolve all *Bordetella* species as very well supported clades (Fig. 3). Moreover, the tree topology of *ompA* based phylogenies was conforming to that of 16S rRNA gene.

Abundance of the sequence data of these two genes of *Bordetella* species in the nucleotide database of GenBank, NCBI is not comparable. In fact, there were only 83 (62 sequences from *B. pertussis* and 21 sequences from other *Bordetella* species) nucleotide sequences of the coding gene for *ompA* belonging to *Bordetella* species. In comparison, there were 247 16S rRNA sequences from *Bordetella* species which were analyzed in our phylogenies (Fig. 1).

16S rRNA based phylogeny showed that there are still some clades in *Bordetella* which seem to be putative undescribed species. However, *ompA* didn't show further data on the diversity and boundaries of the genus which is highly associated with the under-sampling of the nucleotide sequences of this gene (Fig. 3).

#### Discussion

Analyzing the 16S rRNA gene alignment, it was found that this gene, as the main gene in phylogeny purposes in prokaryotes, has some limitations to resolve *Bordetella* species. This weak point of the 16S rRNA gene is very well highlighted in figure. 1, where two of the three most important medical species: *B. bronchiseptica* and *B. parapertussis* were not resolved.

Our results show that the Bordetella species have been mostly detected in soil, water, sediment, and even associated to some plants, worldwide. Further, considering Fig. 1, it is shown that human/animal-associated Bordetella species scatter in the phylogenetic tree of the genus and it is contrary to the results of Soumana et al. (44). Furthermore, phylogenies conducted in this study indicated that the Bordetella species with in some basal positions to the rest of the genus (B.bronchialis, B. flabilis, B. sputigena) have been exclusively detected in human respiratory specimens (24). Thus, the conclusion that the basal clades harbor species with environmental origins is still discussed and it may be in contrary to conclusion of Soumana et al. (2017) (44). According to the data summarized in table 1, the above mentioned species have larger genomes comparing to the rest of the genus. Of course, phylogenies conducted in this study are conforming to those of Vandamme et al. (2015) as these three species have a different node from the other Bordetella species (23). Thus, more taxonomic revisions seems plausible. According to the recent 16S rRNA-based phylogenies, it was assumed that Bordetella species with environmental origins tend to have basal placements in comparison to human/animalspecies (44), but the gigantic associated phylogenies performed here and the results of Vandamme et al. (2015) does not show such a relationship between the origin of the Bordetella species/isolates and their evolutionary placements (23). Also, our phylogenies showed that there are a considerably higher genetic diversity in the basal taxa of the phylogenetic tree of Bordetella which is conforming to the results of Soumana et al. (44). However, considering the documented genome decay rates in Bordetella species, an evolutionary link between species with a free-living environmental lifestyle and the species with a hostrestricted obligately pathogenic lifestyle is probable.

## Conclusion

As a conclusion, considering the analyses performed on the nucleotide sequences of the coding gene for *ompA* a higher resolution achieved for *Bordetella* species. Also, due to the same topologies observed for 16S rRNA and *ompA* genes it is concluded that using coding genes; likely *ompA*, can result more resolutions in *Bordetella* phylogenies which differentiate very close species unequivocally.

## Acknowledgements

This research was supported by Pasteur Institute of Iran (IPI), Tehran, Iran.

## **Conflict of interest**

None declared conflicts of interest.

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