J Med Bacteriol. *Vol. 6, No. 3, 4 (2017): pp.1-13* *jmb.tums.ac.ir*

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

Ali Badamchi [1], *Moslem Papizadeh* [2*]

[1] *Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran.*

[2] *Department of Microbiology, Pasteur Institute of Iran (IPI), Tehran, Iran.*

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 19 Jan 2017
Revised: Jun Mar 2017
Accepted: 11 Sep 2017
Published: 15 Oct 2017

Keywords:
Alcaligenaceae, Biogeography, Bordetella species, Ecological distribution, Phylogenetic species concept.

ABSTRACT

Background: 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.

***Methods*:** In this study, 16S rRNA gene sequence of 16 type strains of the *Bordetella* species were analyzed. Also, phylogenies conducted on the same gene of 247 isolates of *Bordetella* species, comprising a wide physiological as well as ecological diversity and encompassing ex-type representatives of the 16 *Bordetella* species, were analyzed.

***Results*:** 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 promising bootstrap and posterior probability values as our phylogenies, using maximum likelihood and Bayesian inference methods, showed.

***Conclusion*:** Our results indicate a probable need for additional phylogenetic signals which can be provided by coding genes. Therefore, sequence data of *ompA* gene of *Bordetella* 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 *Bordetella* 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.

*Corresponding Author: Moslem Papizadeh, Department of Microbiology, Pasteur Institute of Iran (IPI), Tehran, Iran.

Tel: +98- 026-92108983, +98- 021-64112247, *E-mail:* m_papizadeh@pasteur.ac.ir

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 non-pathogenic 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 animal-pathogenic 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.

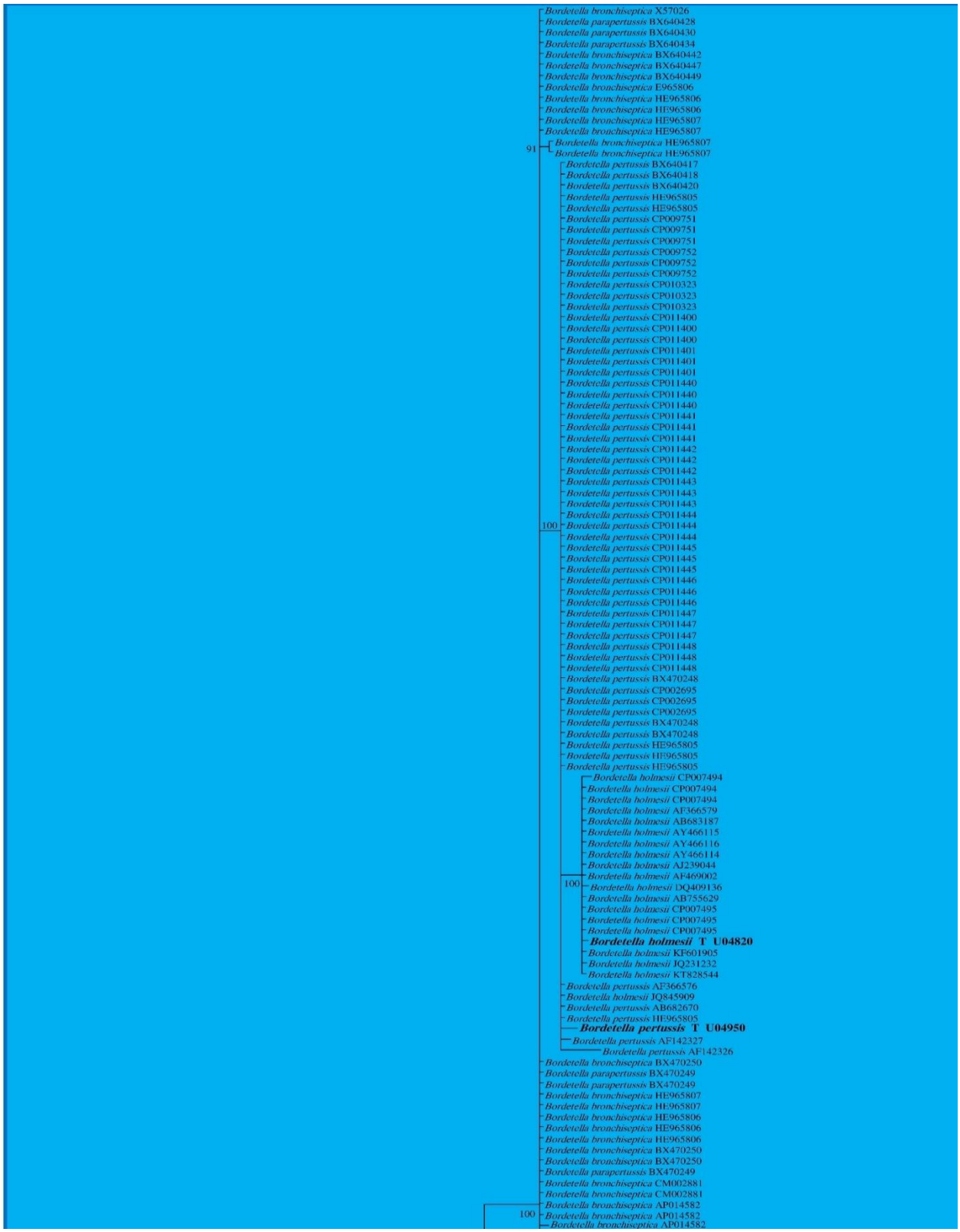
Materials and Methods

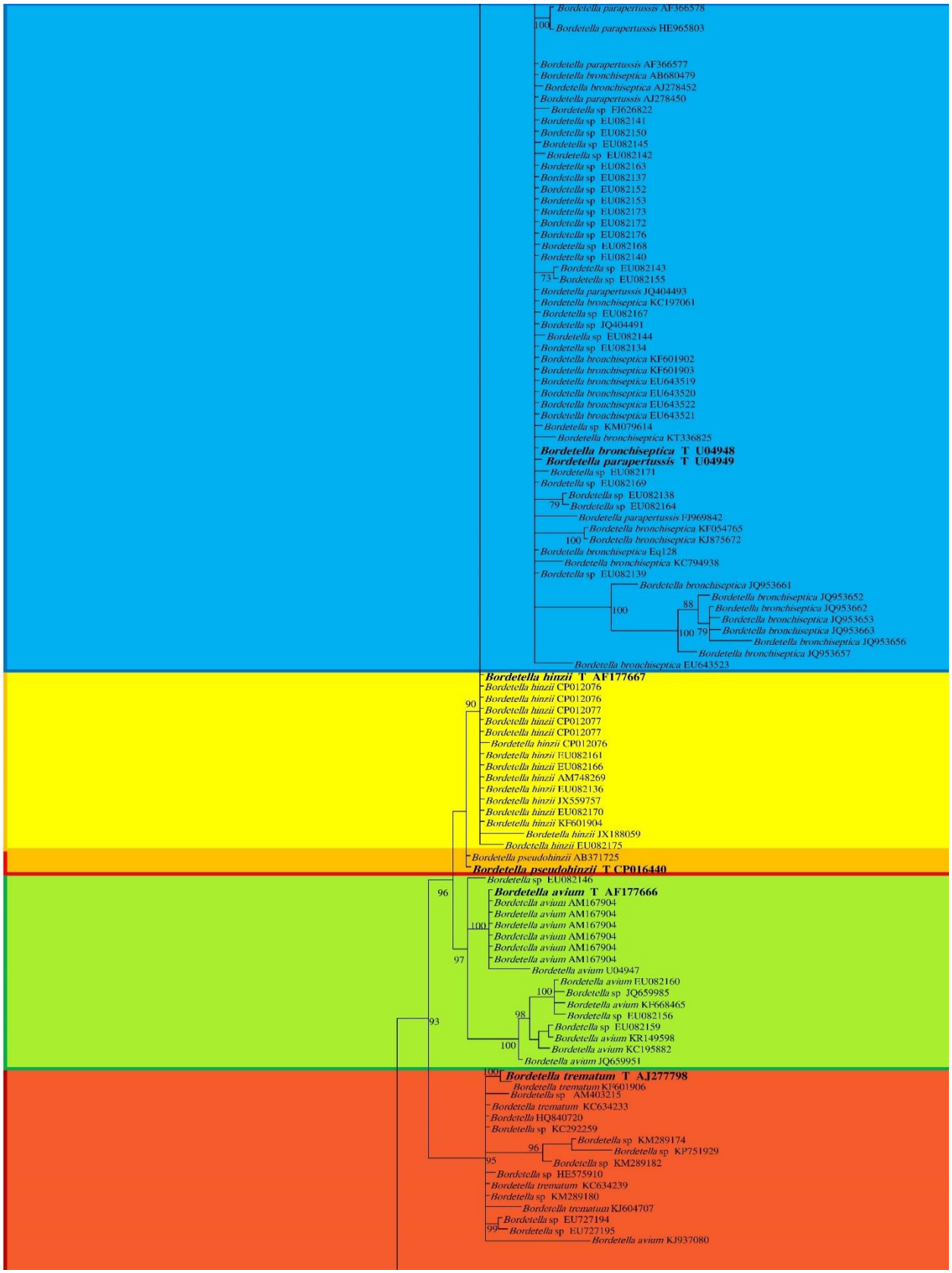
The 16S rRNA gene reference sequences and either the same gene sequences from isolates and uncultured materials obtained from Ribosomal Database Project (27, 28). Also, *ompA* gene sequences obtained from the nucleotide database of GenBank, NCBI. Thus, three different datasets (two datasets for 16S rRNA 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 at the European 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 50000000, 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 alignment which contained the reference sequences for the genera of Alcaligenaceae and allied families.


Bordetella bronchiseptica X57026
Bordetella parapertussis BX640428
Bordetella parapertussis BX640430
Bordetella parapertussis BX640434
Bordetella bronchiseptica BX640442
Bordetella bronchiseptica BX640447
Bordetella bronchiseptica BX640449
Bordetella bronchiseptica E965806
Bordetella bronchiseptica HE965806
Bordetella bronchiseptica HE965806
Bordetella bronchiseptica HE965807
Bordetella bronchiseptica HE965807
91
Bordetella bronchiseptica HE965807
Bordetella bronchiseptica HE965807
Bordetella pertussis BX640417
Bordetella pertussis BX640418
Bordetella pertussis BX640420
Bordetella pertussis HE965805
Bordetella pertussis HE965805
Bordetella pertussis CP009751
Bordetella pertussis CP009751
Bordetella pertussis CP009751
Bordetella pertussis CP009752
Bordetella pertussis CP009752
Bordetella pertussis CP009752
Bordetella pertussis CP010323
Bordetella pertussis CP010323
Bordetella pertussis CP010323
Bordetella pertussis CP011400
Bordetella pertussis CP011400
Bordetella pertussis CP011400
Bordetella pertussis CP011401
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
Bordetella pertussis CP011443
Bordetella pertussis CP011443
Bordetella pertussis CP011443
Bordetella pertussis CP011444
100
Bordetella pertussis CP011444
Bordetella pertussis CP011444
Bordetella pertussis CP011445
Bordetella pertussis CP011445
Bordetella pertussis CP011445
Bordetella pertussis CP011446
Bordetella pertussis CP011446
Bordetella pertussis CP011446
Bordetella pertussis CP011447
Bordetella pertussis CP011447
Bordetella pertussis CP011447
Bordetella pertussis CP011448
Bordetella pertussis CP011448
Bordetella pertussis CP011448
Bordetella pertussis BX470248
Bordetella pertussis CP002695
Bordetella pertussis CP002695
Bordetella pertussis CP002695
Bordetella pertussis BX470248
Bordetella pertussis BX470248
Bordetella pertussis HE965805
Bordetella pertussis HE965805
Bordetella pertussis HE965805
Bordetella holmesii CP007494
Bordetella holmesii CP007494
Bordetella holmesii CP007494
Bordetella holmesii AF366579
Bordetella holmesii AB683187
Bordetella holmesii AY466115
Bordetella holmesii AY466116
Bordetella holmesii AY466114
Bordetella holmesii AJ239044
Bordetella holmesii AF469002
100
Bordetella holmesii DQ409136
Bordetella holmesii AB755629
Bordetella holmesii CP007495
Bordetella holmesii CP007495
Bordetella holmesii CP007495
Bordetella holmesii T U04820
Bordetella holmesii KF601905
Bordetella holmesii JQ231232
Bordetella holmesii KT828544
Bordetella pertussis AF366576
Bordetella holmesii JQ845909
Bordetella pertussis AB682670
Bordetella pertussis HE965805
Bordetella pertussis T U04950
Bordetella pertussis AF142327
Bordetella pertussis AF142326
Bordetella bronchiseptica BX470250
Bordetella parapertussis BX470249
Bordetella parapertussis BX470249
Bordetella bronchiseptica HE965807
Bordetella bronchiseptica HE965807
Bordetella bronchiseptica HE965806
Bordetella bronchiseptica HE965806
Bordetella bronchiseptica HE965806
Bordetella bronchiseptica BX470250
Bordetella bronchiseptica BX470250
Bordetella parapertussis BX470249
Bordetella bronchiseptica CM002881
Bordetella bronchiseptica CM002881
Bordetella bronchiseptica AP014582
100
Bordetella bronchiseptica AP014582
Bordetella bronchiseptica AP014582


Bordetella parapertussis AF366578
Bordetella parapertussis HE965803
Bordetella parapertussis AF366577
Bordetella bronchiseptica AB680479
Bordetella bronchiseptica AJ278452
Bordetella parapertussis AJ278450
Bordetella sp FJ626822
Bordetella sp EU082141
Bordetella sp EU082150
Bordetella sp EU082145
Bordetella sp EU082142
Bordetella sp EU082163
Bordetella sp EU082137
Bordetella sp EU082152
Bordetella sp EU082153
Bordetella sp EU082173
Bordetella sp EU082172
Bordetella sp EU082176
Bordetella sp EU082168
Bordetella sp EU082140
Bordetella sp EU082143
Bordetella sp EU082155
Bordetella parapertussis JQ404493
Bordetella bronchiseptica KC197061
Bordetella sp EU082167
Bordetella sp JQ404491
Bordetella sp EU082144
Bordetella sp EU082134
Bordetella bronchiseptica KF601902
Bordetella bronchiseptica KF601903
Bordetella bronchiseptica EU643519
Bordetella bronchiseptica EU643520
Bordetella bronchiseptica EU643522
Bordetella bronchiseptica EU643521
Bordetella sp KM079614
Bordetella bronchiseptica KT336825
Bordetella bronchiseptica T U04948
Bordetella parapertussis T U04949
Bordetella sp EU082171
Bordetella sp EU082169
Bordetella sp EU082138
Bordetella sp EU082164
Bordetella parapertussis FJ969842
Bordetella bronchiseptica KF054765
Bordetella bronchiseptica KJ875672
Bordetella bronchiseptica Eq128
Bordetella bronchiseptica KC794938
Bordetella sp EU082139
Bordetella bronchiseptica JQ953661
Bordetella bronchiseptica JQ953652
Bordetella bronchiseptica JQ953662
Bordetella bronchiseptica JQ953653
Bordetella bronchiseptica JQ953663
Bordetella bronchiseptica JQ953656
Bordetella bronchiseptica JQ953657
Bordetella bronchiseptica EU643523
Bordetella hinzii T AF177667
Bordetella hinzii CP012076
Bordetella hinzii CP012076
Bordetella hinzii CP012077
Bordetella hinzii CP012077
Bordetella hinzii CP012077
Bordetella hinzii CP012076
Bordetella hinzii EU082161
Bordetella hinzii EU082166
Bordetella hinzii AM748269
Bordetella hinzii EU082136
Bordetella hinzii JX559757
Bordetella hinzii EU082170
Bordetella hinzii KF601904
Bordetella hinzii JX188059
Bordetella hinzii EU082175
Bordetella pseudohinzii AB371725
Bordetella pseudohinzii T CP016440
Bordetella sp EU082146
Bordetella avium T AF177666
Bordetella avium AM167904
Bordetella avium AM167904
Bordetella avium AM167904
Bordetella avium AM167904
Bordetella avium AM167904
Bordetella avium AM167904
Bordetella avium U04947
Bordetella avium EU082160
Bordetella sp JQ659985
Bordetella avium KF668465
Bordetella sp EU082156
Bordetella sp EU082159
Bordetella avium KR149598
Bordetella avium KC195882
Bordetella avium JQ659951
Bordetella trematum T AJ277798
Bordetella trematum KF601906
Bordetella sp AM403215
Bordetella trematum KC634233
Bordetella HQ840720
Bordetella sp KC292259
Bordetella sp KM289174
Bordetella sp KP751929
Bordetella sp KM289182
Bordetella sp HE575910
Bordetella trematum KC634239
Bordetella sp KM289180
Bordetella trematum KJ604707
Bordetella sp EU727194
Bordetella sp EU727195
Bordetella avium KJ937080

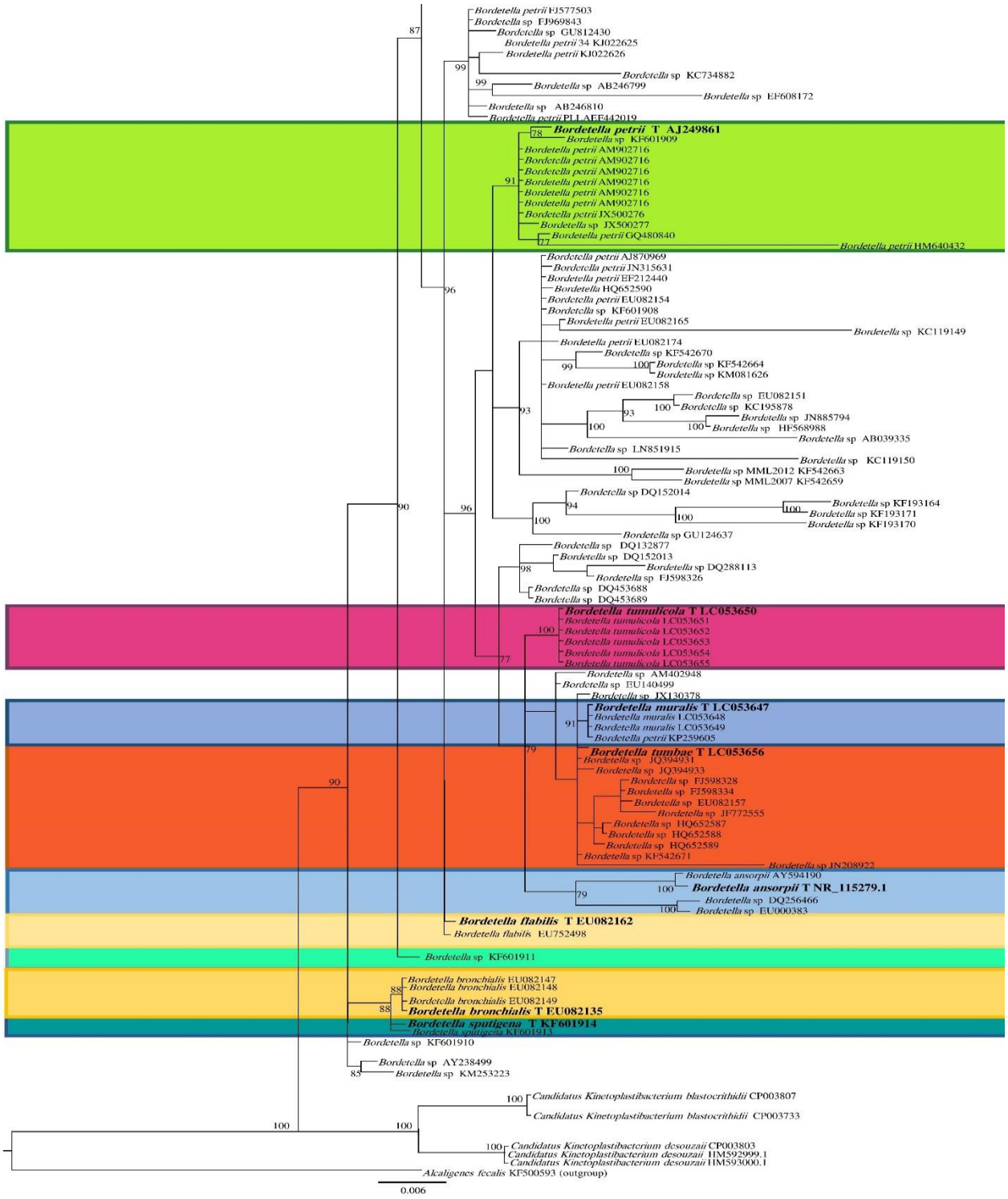


Figure 1. The Bayesian inference phylogeny of the members of the genus *Bordetella* based on the 16S rRNA gene sequence data. Bayesian posterior probabilities above 0.75 resulting from 50,000,000 replicates are given at the nodes. The GenBank accessions are given after the species names. Species are 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*.

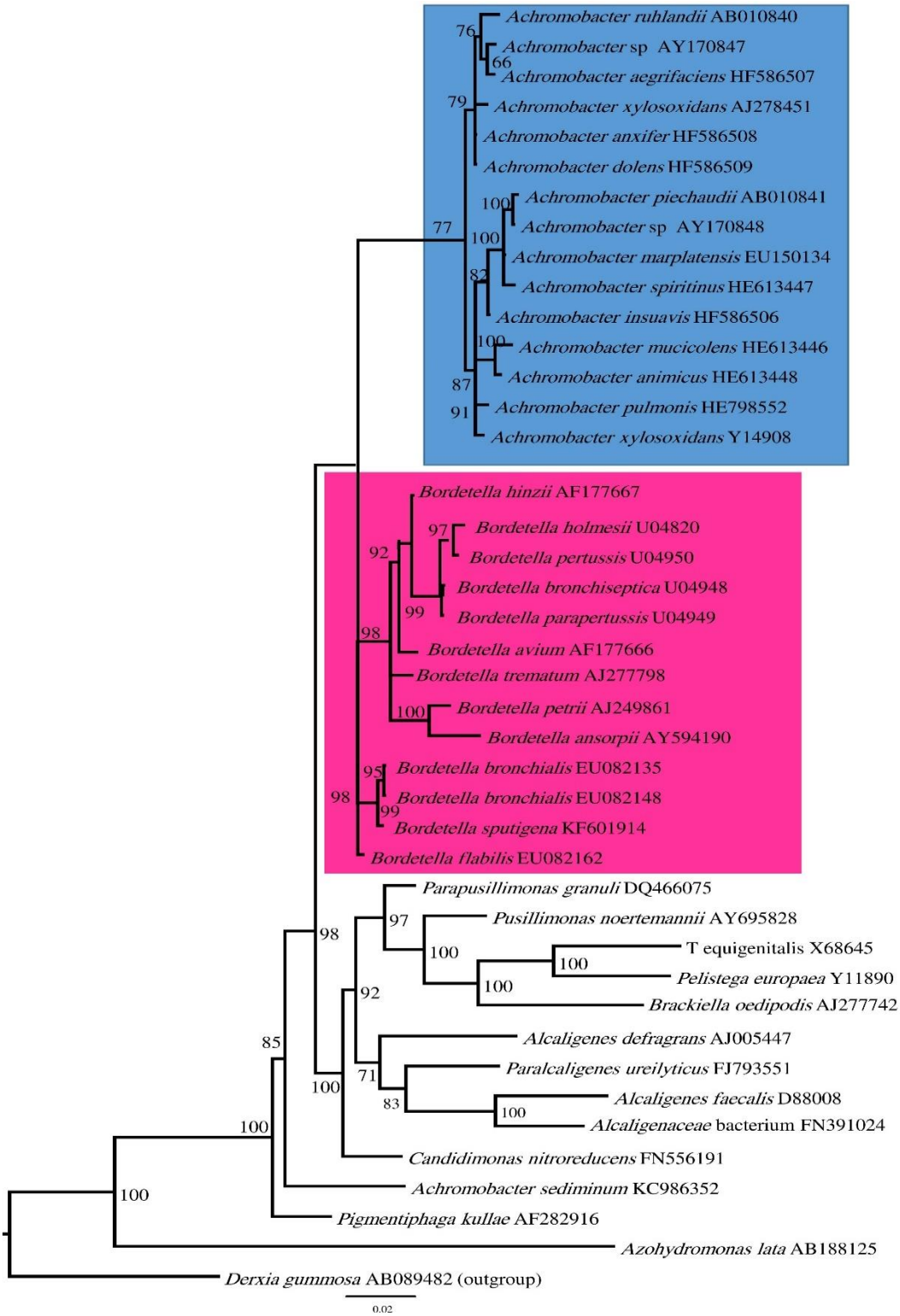
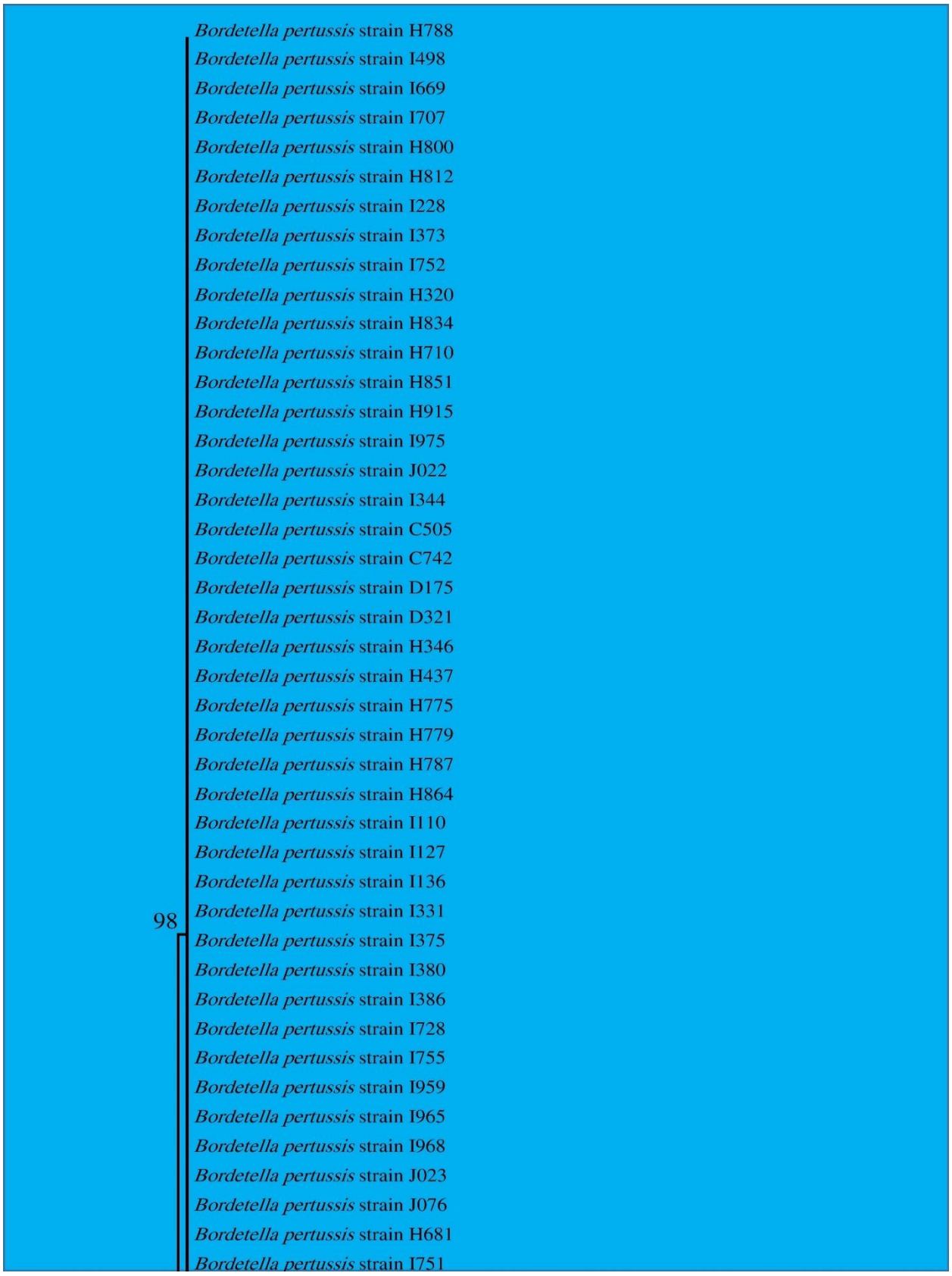


Figure 2. The Bayesian inference phylogeny of the genus *Bordetella* based on the 16S rRNA gene sequence data of type strains. Bayesian posterior probabilities above 0.75 resulting from 20,000,000 replicates are given at the nodes. The GenBank accessions are given after the species names. The genera *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 I669
Bordetella pertussis strain I707
Bordetella pertussis strain H800
Bordetella pertussis strain H812
Bordetella pertussis strain I228
Bordetella pertussis strain I373
Bordetella pertussis strain I752
Bordetella pertussis strain H320
Bordetella pertussis strain H834
Bordetella pertussis strain H710
Bordetella pertussis strain H851
Bordetella pertussis strain H915
Bordetella pertussis strain I975
Bordetella pertussis strain J022
Bordetella pertussis strain I344
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
98
Bordetella pertussis strain I331
Bordetella pertussis strain I375
Bordetella pertussis strain I380
Bordetella pertussis strain I386
Bordetella pertussis strain I728
Bordetella pertussis strain I755
Bordetella pertussis strain I959
Bordetella pertussis strain I965
Bordetella pertussis strain I968
Bordetella pertussis strain J023
Bordetella pertussis strain J076
Bordetella pertussis strain H681
Bordetella pertussis strain I751

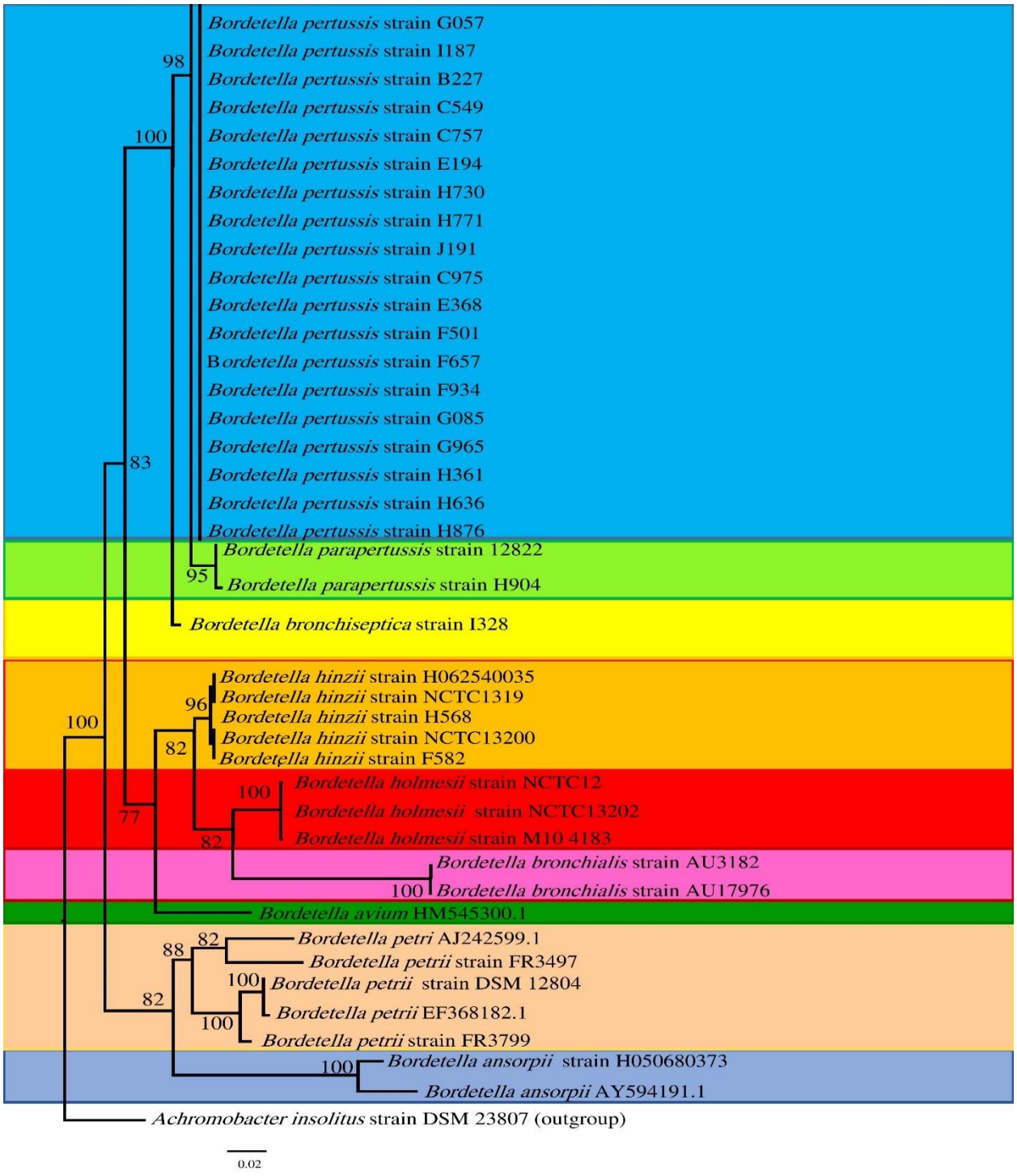


Figure 3. The Bayesian inference phylogeny of the members of the genus *Bordetella* based on the sequence data of the coding gene for *ompA*. Bayesian posterior probabilities above 0.75 resulting from 20,000,000 replicates are given at the nodes. The GenBank accessions are given after the species names. 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 assemblies	Median total length (Mb)	Median protein count	Median GC content (%)
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/animal-associated species (44), but the gigantic 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 host-restricted 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.

References

1. Rahimi F, Talebi M, Saifi M, Pourshafie MR. Distribution of enterococcal species and detection of vancomycin resistance genes by multiplex PCR in Tehran sewage. *Iran Biomed J* 2007; **11**(3): 161–7.
2. Talebi, M., F. Rahimi, M. Katouli, I. et al. 2007. Prevalence and antimicrobial resistance of enterococcal species in sewage treatment plants in Iran. *Water Air Soil Pollut* 2007; **185**: 111–119.
3. Papizadeh M, Roayaei Ardakani M, Motamedi H (2017) Growth-phase dependent biodesulfurization of dibenzothiophene by *Enterobacter* sp. strain NISOC-03. *Pollution* **3**(1):101–111.
4. Papizadeh M, Roayaei Ardakani M, Ebrahimipour G, et al. (2010) Utilization of dibenzothiophene as sulfur source by *Microbacterium* sp. NISOC-06. *World J Microbiol Biotechnol* **26**:1195–1200.
5. Papizadeh M, Roayaei Ardakani M, Motamedi H, et al. (2011) C-S targeted biodegradation of dibenzothiophene by *Stenotrophomonas* sp. NISOC-04. *Appl Biochem Biotechnol* **165**: 938–948.
6. Papizadeh M, Roayaei Ardakani M (2010). Bio filtration of volatile sulphurous hydrocarbon-polluted air by hydrocarbon degrading *Pseudomonas* NISOC-11. *J Biotechnol* **150**:209–210.
7. Gerlach G, von Wintzingerode F, Middendorf B, et al. Evolutionary trends in the genus *Bordetella*. *Microb Infect* 2001; **3**(1): 61–72.
8. von Wintzingerode F, Schattke A, Siddiqui RA, et al. *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. *IJSEM* 2001 **51**(4): 1257–65.
9. Lehmann PF. PR Murray, EJ Baron, et al,

eds. Manual of clinical microbiology. *Mycopathologia* 1999; **146**(2): 107–8.
10. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. *Clin Microbiol Rev* 2005; **18**(2): 326–82.
11. Register KB, Ivanov YV, Harvill ET, et al. Novel, host-restricted genotypes of *Bordetella bronchiseptica* associated with phocine respiratory tract isolates. *Microbiology* 2015; **161**(3): 580–92.
12. Cummings CA, Brinig MM, Lepp PW, et al. *Bordetella* species are distinguished by patterns of substantial gene loss and host adaptation. *J Bacteriol* 2004; **186**(5): 1484–92.
13. Vandamme P, Hommez J, Vancanneyt M, et al. *Bordetella hinzii* sp. nov., isolated from poultry and humans. *IJSEM*. 1995; **45**(1): 37–45.
14. Vandamme P, Heyndrickx M, Vancanneyt M, et al. *Bordetella trematum* sp. nov., isolated from wounds and ear infections in humans, and reassessment of *Alcaligenes denitrificans* Rüger and Tan 1983. *IJSEM* 1996; **46**(4): 849–58.
15. Tang YW, Hopkins MK, Kolbert CP, et al. *Bordetella holmesii*-like organisms associated with septicemia, endocarditis, and respiratory failure. *Clin Infect Dis* 1998; **26**(2): 389–92.
16. Weyant RS, Hollis DG, Weaver RE, et al. *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. *J Clin Microbiol* 1995; **33**(1): 1–7.
17. Abouanaser SF, Srigley JA, Nguyen T, et al. *Bordetella holmesii*, an emerging cause of septic arthritis. *J Clin Microbiol* 2013; **51**(4): 1313–5.
18. Nagata JM, Charville GW, Klotz JM, et al. *Bordetella petrii* sinusitis in an immunocompromised adolescent. *Pediatr Infect Dis J* 2015; **34**(4): 458.
19. Kattar MM, Chavez JF, Limaye AP, et al. Application of 16S rRNA gene sequencing to identify *Bordetella hinzii* as the causative agent of fatal septicemia. *J Clin Microbiol* 2000; **38**(2): 789–94.
20. Fry NK, Duncan J, Edwards MT, et al. A UK clinical isolate of *Bordetella hinzii* from a patient with myelodysplastic syndrome. *J Clin Microbiol* 2007; **56**(12): 1700–3.
21. Vandamme P, Heyndrickx M, Vancanneyt M, et al. *Bordetella trematum* sp. nov., isolated from wounds and ear infections in humans, and reassessment of *Alcaligenes denitrificans* Rüger and Tan 1983. *IJSEM* 1996; **46**(4): 849–58.
22. Ko KS, Peck KR, Oh WS, et al. New species of *Bordetella*, *Bordetella ansorpii* sp. nov., isolated from the purulent exudate of an epidermal cyst. *J Clin Microbiol* 2005; **43**(5): 2516–9.
23. Spilker T, Darrah R, LiPuma JJ. Complete genome sequences of *Bordetella flabilis*, *Bordetella bronchialis*, and *Bordetella pseudohinzii*. *Genome Announc* 2016; **4**(5): e01132–16.
24. Vandamme PA, Peeters C, Cnockaert M, et al. *Bordetella bronchialis* sp. nov., Bordetella flabilis sp. nov. and *Bordetella sputigena* sp. nov., isolated from human respiratory specimens, and reclassification of *Achromobacter sediminum* Zhang et al. 2014 as *Verticia sediminum* gen. nov., comb. nov. *IJSEM*. 2015; **65**(10): 3674–82.
25. Ivanov YV, Linz B, Register KB, et al. Identification and taxonomic characterization of *Bordetella pseudohinzii* sp. nov. isolated from laboratory-raised mice. *IJSEM* 2016; **66**(12): 5452–9.
26. Saba F, Papizadeh M, Khansha J, et al. A

rapid and reproducible genomic dna extraction protocol for sequence-based identification of archaea, bacteria, cyanobacteria, diatoms, fungi, and green algae. *J Med bacteriol* 2017; **5**(3-4): 22–8.
27. Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol biol evol* 2013; **30**(12): 2725–9.
28. Cole JR, Wang Q, Fish JA, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic acids res* 2013: gkt1244.
29. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol biol evol* 2013; **30**(4): 772–80.
30. Katoh K, Asimenos G, Toh H (2009) Multiple alignment of DNA sequences with MAFFT. *Methods Mol Biol* 2009; **537**: 39–64.
31. McWilliam H, Li W, Uludag M, et al. Analysis tool web services from the EMBL-EBI. *Nucleic acids res* 2013; **41**(W1): W597–600.
32. Papizadeh M, Soudi MR, Amini L, et al. *Pyrenochaetopsis tabarestanensis* (Cucurbitariaceae, Pleosporales), a new species isolated from rice farms in north Iran. *Phytotaxa* 2017; **297**(1): 15–28.
33. Papizadeh M, Wijayawardene NN, Amoozegar MA, et al. *Neocamarosporium jorjanensis*, *N. persepolisi*, and *N. solicola* spp. nov. (Neocamarosporiaceae, Pleosporales) isolated from saline lakes of Iran indicate the possible halotolerant nature for the genus. *Mycol Progress* 2017; 1-19.
34. Papizadeh M, Rohani M, Nahrevanian H, et al. Probiotic characters of *Bifidobacterium* and *Lactobacillus* are a result of the ongoing gene acquisition and genome minimization evolutionary trends. *Microb Pathog* 2017; 111: 118–31.
35. Papizadeh M, Pourshafie MR. Niche-specific genome evolution in gastrointestinal probiotics. *Biomed J Sci & Tech Res* 1 (3).
36. Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; **28**(10): 2731–9.
37. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016:msw054.
38. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 2001; **17**(8): 754–5.
39. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Gateway Computing Environments Workshop (GCE), 2010 2010 (pp. 1-8). Ieee.
40. Nylander JA, Ronquist F, Huelsenbeck JP, et al. Bayesian phylogenetic analysis of combined data. *Syst Biol* 2004; **53**(1): 47–67.
41. Lemey P, Rambaut A, Drummond AJ, et al. Bayesian phylogeography finds its roots. *PLoS Comput Biol* 2009; **5**(9): e1000520.
42. Guo D, Shibuya R, Akiba C, Saji S, Kondo T, Nakamura J. Active sites of nitrogen-doped carbon materials for oxygen reduction reaction clarified using model catalysts. *Science* 2016; **351**(6271): 361–5.
43. Rambaut A, Pybus OG, Nelson MI, et al. The genomic and epidemiological dynamics of human influenza A virus. *Nature*. 2008; **453**(7195): 615–9.
44. Soumana IH, Linz B, Harvill ET. Environmental origin of the genus *Bordetella*. *Front Microbiol* 2017; **8**: 28.