

References

1. Boisier P, Nicolas P, Djibo S, Taha MK, Jeanne I, Mainassara HB, et al. Meningococcal meningitis: unprecedented incidence of serogroup X-related cases in 2006 in Niger. *Clin Infect Dis*. 2007; 44:657–63. <http://dx.doi.org/10.1086/511646>
2. Delrieu I, Yaro S, Tameklóé TA, Njanpop-Lafourcade BM, Tall H, Jaillard P, et al. Emergence of epidemic *Neisseria meningitidis* serogroup X meningitis in Togo and Burkina Faso. *PLoS One*. 2011;6:e19513. <http://dx.doi.org/10.1371/journal.pone.0019513>
3. Fazio C, Starnino S, Dal Solda M, Sofia T, Neri A, Mastrantonio P, et al. *Neisseria meningitidis* serogroup X sequence type 2888, Italy. *Emerg Infect Dis*. 2010;16:359–60. <http://dx.doi.org/10.3201/eid1602.091553>
4. Vicente D, Esnal O, Pérez-Trallero E. Fatal *Neisseria meningitidis* serogroup X sepsis in immunocompromised patients in Spain. Virulence of clinical isolates. *J Infect*. 2012;64:184–7. <http://dx.doi.org/10.1016/j.jinf.2011.11.009>
5. Pan J, Yao P, Zhang H, Sun X, He H, Xie S. The case of a new sequence type 7 serogroup X *Neisseria meningitidis* infection in China: may capsular switching change serogroup profile? *Int J Infect Dis*. 2014;29:62–4. <http://dx.doi.org/10.1016/j.ijid.2014.07.022>
6. Stefanelli P, Fazio C, Neri A, Ciammaruconi A, Balocchini E, Anselmo A, et al. Genome-based study of a spatio-temporal cluster of invasive meningococcal disease due to *Neisseria meningitidis* serogroup C, clonal complex 11. *J Infect*. 2016;73:136–44. <http://dx.doi.org/10.1016/j.jinf.2016.05.003>
7. Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics*. 2010;11:595. <http://dx.doi.org/10.1186/1471-2105-11-595>
8. Agnemesel A, Hong E, Giorgini D, Nuñez-Samudio V, Deghmane AE, Taha MK. *Neisseria meningitidis* Serogroup X in sub-Saharan Africa. *Emerg Infect Dis*. 2016;22:698–702. <http://dx.doi.org/10.3201/eid2204.150653>

Address for correspondence: Paola Stefanelli, Department of Infectious, Parasitic & Immuno-mediated Disease, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy; email: paola.stefanelli@iss.it

Clinical Manifestations of Punta Toro Virus Species Complex Infections, Panama, 2009

Nathan D. Gundacker,¹ Jean-Paul Carrera,¹ Marlene Castillo, Yamilka Díaz, Jose Valenzuela, Ashutosh Tamhane, Brechla Moreno, Juan Miguel Pascale, Robert B. Tesh, Sandra López-Vergès

¹These authors contributed equally to this article.

Authors affiliations: University of Alabama at Birmingham, Birmingham, Alabama, USA (N.D. Gundacker, A. Tamhane); Gorgas Memorial Institute for Health Studies, Panama City, Panama (J.-P. Carrera, M. Castillo, Y. Díaz, J. Valenzuela, B. Moreno, J.M. Pascale, S. López-Vergès); University of Texas Medical Branch, Galveston, Texas, USA (R.B. Tesh).

DOI: <https://dx.doi.org/10.3201/eid2305.161925>

An investigation in Panama found that Punta Toro virus species complex (PTVs) may contribute to febrile illnesses with symptoms mirroring those of dengue fever. However, further studies are needed to determine if PTV infection causes only a mild disease or if it can have more serious manifestations in some patients.

Acute febrile illness in the New World tropics has a broad differential diagnosis largely dependent on locale and seasonal outbreaks. In Central America, most febrile illnesses have historically been attributed to dengue or malaria. However, recent evidence from Panama suggests varied differential diagnoses, including hantavirus, chikungunya virus, and Zika virus infection (1,2). In 2009, a dengue outbreak was reported in Panama City, Panama. The Gorgas Memorial Institute in Panama City tested dengue-negative samples from this outbreak for alphaviruses, flaviviruses, and phleboviruses and detected Punta Toro virus species complex (PTVs) in some samples. PTV (genus *Phlebovirus*, family *Bunyaviridae*), a member of the sand fly fever group, was initially described in humans in 1966 after being isolated from a soldier in Panama who had fever, headache, myalgia, and leukopenia (3). The phylogenetics of PTV have been thoroughly characterized (4–6), but our search of the literature did not reveal reports of other PTV cases in humans.

The signs and symptoms of sand fly-associated phlebovirus infection vary, but most infections cause a mild febrile illness characterized by retroorbital headache, weakness, back pain, and leukopenia. However, infection with 2 other phleboviruses, mosquito-borne Rift Valley fever virus and tick-associated severe fever with thrombocytopenia syndrome virus, causes severe disease. Little is known regarding the signs, symptoms, and clinical course of PTV infection in humans.

During the 2009 investigation, the Gorgas Memorial Institute analyzed 4,852 samples from persons in Panama with suspected acute dengue; 1,667 (34.4%) of the samples were dengue-negative. We further analyzed 201 of these samples for phlebovirus (online Technical Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/23/5/16-1925-Techapp1.pdf>). In brief, we extracted viral RNA from the samples and evaluated it by using *Phlebovirus* genus-specific reverse transcription PCR (RT-PCR) based on the

highly conserved L (large) gene that detects Toscana, Naples, Sicilian, Aguacate, Punta Toro, and Rift Valley fever viruses (7). We also screened samples using panflavivirus and panalphavirus RT-PCRs.

Of the 201 samples, 27 (13.4%) were RT-PCR-positive for phlebovirus. BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) nucleotide sequence comparison suggested all were PTVs; 1 was previously described as Cocle virus (4). We conducted phylogenetic analyses on 11 of the phlebovirus-positive samples, using a 482-nt sequence and MEGA7 software (8). An optimal maximum-likelihood tree confirmed the samples were PTVs; all samples from 2009 (GenBank accession nos. KY43355–KY435365) clustered together close to Cocle virus (online Technical Appendix Table 2) (4). Our attempts to isolate virus were unsuccessful. To determine if PTV had been previously detected, we tested 202 randomly selected dengue virus-negative samples from 2008; none was phlebovirus-positive.

Clinical data sheets were available for 92.6% (25/27) of the PTV-positive samples. After de-identifying the data, we entered it into a dataset. A control group consisted of 90 dengue virus-positive patients from 2009 who were frequency matched by age and randomly selected from available records. The PTV-positive case-patients were largely located in the Panama City metropolitan area (Figure). Case-patients and controls were compared primarily with regard to reported symptoms (online Technical Appendix Table 3). Case-patients were significantly less likely than controls to have exanthema (22% vs. 54%; odds ratio 0.23, 95% CI 0.08–0.66; $p = 0.01$). We found no major clinical differences between case-patients and controls with regard

to other symptoms. No patients in either group had shock or hemorrhagic syndrome.

Febrile syndromes in the tropics are often treated empirically; clinical decisions are often made on the basis of epidemiologic information and concurrent outbreaks. In Central America, dengue fever and malaria are treated without confirmatory testing because testing is costly and time-consuming. However, an increasing number of agents responsible for causing febrile illnesses have been identified in recent years. The variety of clinical outcomes observed with hantavirus and dengue, chikungunya, and Zika virus infections underscores the need for more accurate diagnostics to differentiate between causative agents. Clinical decisions must rely on accurate diagnoses because symptomatology is not an accurate predictor of the true etiology of a febrile illness.

Our findings suggest that, in Panama, PTVs may be a major contributor to febrile illnesses with symptoms mirroring those of dengue fever. However, the clinical course and range of disease caused by PTVs are unknown. Prospective studies are needed to determine if PTV infection causes only mild disease or if it can have serious manifestations in some patients.

PTVs are assumed to be sand fly-borne, and sand flies are usually present in rural or forested areas (9). However, most cases of PTVs infection in Panama in 2009 were in urban and periurban areas, raising questions about the vector, the vector's habitat, and the mode of virus transmission. Panama City, however, is home to two thirds of the country's population and has improved healthcare infrastructure, which may explain the higher number of confirmatory

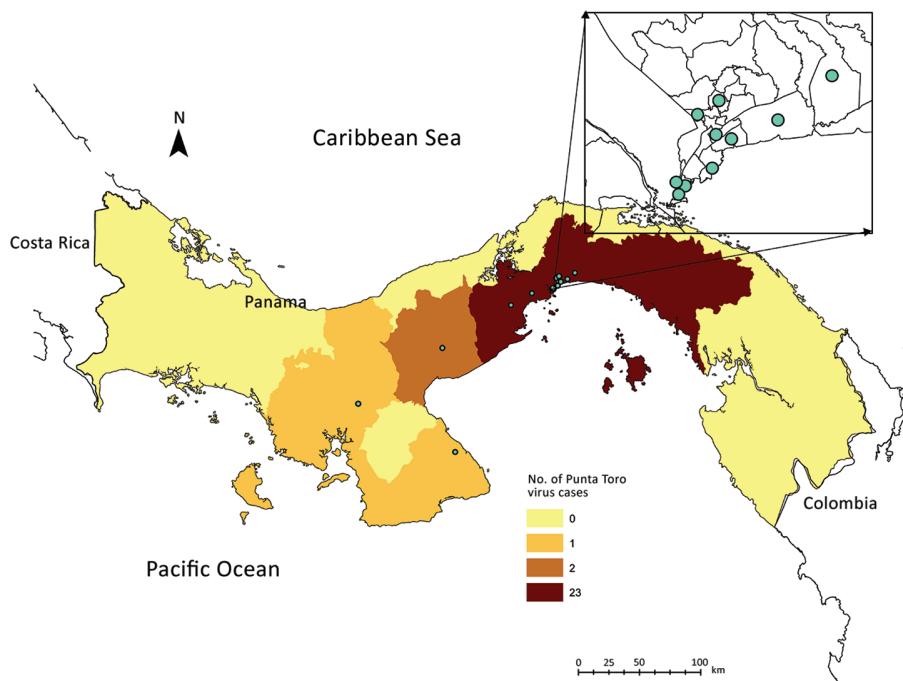


Figure. Distribution of confirmed Punta Toro species complex infections, Panama, 2009. Dots indicate cases. Inset shows enlargement of Panama City area.

tests from Panama City versus other areas of Panama and might result in a sampling bias. Despite these limitations, the recent Zika outbreak has shown the speed at which vectorborne diseases can spread and highlights the importance of detecting emerging viruses like PTVs.

Acknowledgments

We thank staff in the Department of Research in Virology and Biotechnology at the Gorgas Memorial Institute for Health Studies in Panama and in the Ministry of Health National Epidemiology Department for the surveillance data and outbreak response during 2009. We also thank Meghan Tipre for help creating the epidemiologic map.

This work was done in compliance with the Gorgas Bioethics Committee (1010/CBI/ICGES/15).

Funding was provided by the Panama Ministry of Economy and Finance (09.044.051 to S.L.-V. and 09.044.050 to J.M.P.); the Secretaría Nacional de Ciencia, Tecnología, e Innovación (SENACYT; FID-09-103 to J.-P.C); and Gorgas Memorial Institute, University of Alabama at Birmingham (to N.D.G.). J.M.P. and S.L.-V. are members of the Sistema Nacional de Investigación (SNI) of SENACYT in Panama.

Dr. Gundacker is an infectious disease fellow at the University of Alabama at Birmingham. His primary interest are the clinical description of febrile tropical infectious diseases, laboratory differential diagnosis of these diseases, and host–pathogen interactions. Mr. Carrera is an epidemiologist and virologist at Gorgas Memorial Institute. His primary research interests are ecology, evolution, and epidemiology of arthropodborne and zoonotic viruses.

References

1. Arminen B, Pascale JM, Munoz C, Lee SJ, Choi KL, Avila M, et al. Incidence rate for hantavirus infections without pulmonary syndrome, Panama. *Emerg Infect Dis*. 2011;17:1936–9. <http://dx.doi.org/10.3201/eid1710.101717>
2. Fauci AS, Morens DM. Zika virus in the Americas—yet another arbovirus threat. *N Engl J Med*. 2016;374:601–4. <http://dx.doi.org/10.1056/NEJMp1600297>
3. Tesh RB, Chaniotis BN, Peralta PH, Johnson KM. Ecology of viruses isolated from Panamanian phlebotomine sandflies. *Am J Trop Med Hyg*. 1974;23:258–69.
4. Palacios G, Wiley MR, Travassos da Rosa AP, Guzman H, Quiroz E, Savji N, et al. Characterization of the Punta Toro species complex (genus *Phlebotomus*, family *Buniviridae*). *J Gen Virol*. 2015;96:2079–85. <http://dx.doi.org/10.1099/vir.0.000170>
5. Xu F, Chen H, Travassos da Rosa AP, Tesh RB, Xiao SY. Phylogenetic relationships among sandfly fever group viruses (*Phlebotomus*: *Buniviridae*) based on the small genome segment. *J Gen Virol*. 2007;88:2312–9. <http://dx.doi.org/10.1099/vir.0.82860-0>
6. Liu DY, Tesh RB, Travassos Da Rosa AP, Peters CJ, Yang Z, Guzman H, et al. Phylogenetic relationships among members of the genus *Phlebotomus* (*Buniviridae*) based on partial M segment sequence analyses. *J Gen Virol*. 2003;84:465–73. <http://dx.doi.org/10.1099/vir.0.18765-0>
7. Sánchez-Seco MP, Echevarría JM, Hernández L, Estévez D, Navarro-Marí JM, Tenorio A. Detection and identification of Toscana and other phleboviruses by RT–nested-PCR assays with degenerated primers. *J Med Virol*. 2003;71:140–9. <http://dx.doi.org/10.1002/jmv.10465>
8. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33:1870–4. <http://dx.doi.org/10.1093/molbev/msw054>
9. Valderrama A, Tavares MG, Andrade Filho JD. Anthropogenic influence on the distribution, abundance and diversity of sandfly species (Diptera: Phlebotominae: Psychodidae), vectors of cutaneous leishmaniasis in Panama. *Mem Inst Oswaldo Cruz*. 2011; 106:1024–31. <http://dx.doi.org/10.1590/S0074-02762011000800021>

Address for correspondence: Sandra López-Vergès, Gorgas Memorial Institute for Health Studies, Department of Research in Virology and Biotechnology, Ave Justo Arosemena and St 35, No. 0816-02593, Panama City, Panama; email: slopez@gorgas.gob.pa

mcr-1 Colistin Resistance in ESBL-Producing *Klebsiella pneumoniae*, France

Yvan Caspar, Mylène Maillet, Patricia Pavese, Gilles Francony, Jean-Paul Brion, Marie-Reine Mallaret, Richard Bonnet, Frédéric Robin, Racha Beyrouthy, Max Maurin

Author affiliations: Centre Hospitalier Universitaire Grenoble-Alpes, Grenoble, France (Y. Caspar, M. Maillet, P. Pavese, G. Francony, J.-P. Brion, M.-R. Mallaret, M. Maurin); University Grenoble Alpes, CNRS, Grenoble; Centre Hospitalier Universitaire Clermont-Ferrand, Clermont-Ferrand, France (R. Bonnet, F. Robin, R. Beyrouthy); Centre National de Référence de la Résistance aux Antibiotiques, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy); Université Clermont Auvergne, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy); Institut National de la Santé et de la Recherche Médicale, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy); Institut National de la Recherche Agronomique, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy)

DOI: <http://dx.doi.org/10.3201/eid2305.161942>

We report intestinal carriage of an extended-spectrum β -lactamase–producing *Klebsiella pneumoniae* strain with high-level resistance to colistin (MIC 24 mg/L) in a patient in France who had been hospitalized for fungal meningitis. The strain had the *mcr-1* plasmid gene and an inactivated *mgrB* gene, which are associated with colistin resistance.

Clinical Manifestations of Punta Toro Virus Species Complex Infections, Panama, 2009

Technical Appendix

Technical Appendix Table 1. Location and number of dengue negative samples analyzed for Punta Toro species complex, 2009

Provinces of Panama	DENV negative samples	Number analyzed for Phlebovirus	Percent analyzed per province	Number positive for PTV
Bocas del Toro*	0	0	–	–
Chiriqui	53	6	11.3	0
Cocle	129	13	10.1	2
Colon	12	2	16.7	0
Darien†	15	0	0.0	–
Herrera	17	2	11.8	0
Kuna Yala	5	1	20.0	0
Los Santos	44	7	15.9	1
Ngobe Bugle‡	1	0	0.0	–
Panama Este	31	3	9.7	0
Panama Metro	691	99	14.3	18
Panama Oeste	172	17	9.9	3
San Miguelito	433	44	10.2	2
Veraguas	53	6	11.3	1
Total	1667	201	12.1	27‡

*No samples received from Bocas del Toro Region.

†Samples from Darien and Ngobe Bugle were lost before analysis.

‡From the 27 samples positive for Phlebovirus, one was Cocle virus previously described (1), and the other 26 are described for the first time.

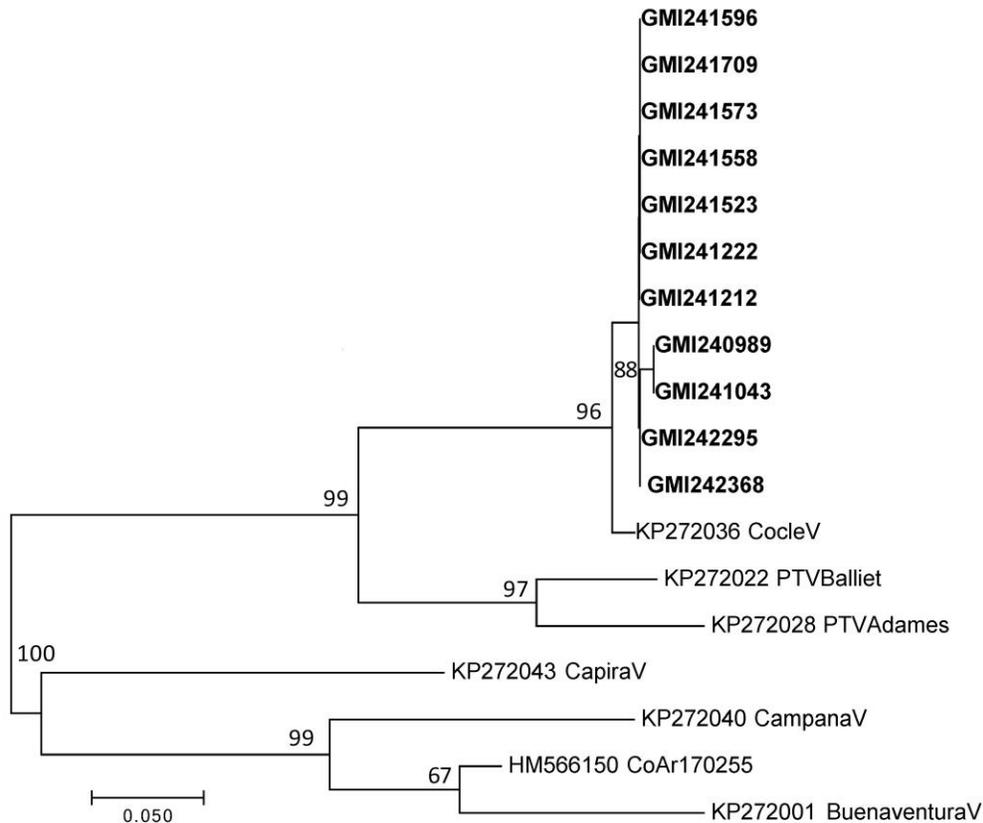
Technical Appendix Table 2. Comparison of demographics and symptoms between Punta Toro species complex and Dengue virus patients*

Characteristic	Overall N (%)	PTV (N = 25) n (%)	DENV (N = 90) n (%)	Odds Ratio (95% CI)†	p-value‡
Demographics					
Age (years) ‡	24 (13–37)	24 (16–37)	22 (12–37)	–	–
Sex					
M	49 (45)	13 (52)	36 (42)	–	–
F	61 (55)	12 (48)	49 (58)	–	–
Symptoms					
Fever	112 (97)	25 (100)	87 (97)	2.04 (0.19–277.74)	0.65
Chills	105 (91)	23 (92)	82 (91)	1.12 (0.22–5.65)	0.89
Severe Headache	104 (90)	22 (88)	82 (91)	0.72 (0.18–2.92)	0.64
Retro-orbital pain	83 (72)	17 (68)	66 (73)	0.77 (0.30–2.02)	0.60
Myalgia	94 (82)	21 (84)	73 (81)	1.22 (0.37–4.03)	0.74
Arthralgia	81 (70)	15 (60)	66 (73)	0.55 (0.22–1.38)	0.20
Exanthem	52 (45)	5 (20)	47 (52)	0.23 (0.08–0.66)	0.01
Cough	24 (21)	8 (32)	16 (18)	2.18 (0.80–5.91)	0.13
Sore Throat	22 (19)	4 (16)	18 (20)	0.76 (0.23–2.50)	0.65
Coryza	13 (11)	3 (12)	10 (11)	1.09 (0.28–4.31)	0.90
Hepatomegaly	3 (3)	0 (0)	3 (3)	0.49 (0.004–5.30)	0.65
Splenomegaly	1 (1)	0 (0)	1 (1)	1.17 (0.01–22.62)	0.93
Nausea/vomiting	49 (43)	9 (36)	40 (44)	0.70 (0.28–1.76)	0.45
Diarrhea	23 (20)	3 (12)	20 (22)	0.48 (0.13–1.76)	0.27
Petechiae	7 (6)	1 (4)	6 (7)	0.58 (0.07–5.08)	0.63

*Bold font indicates the only symptom that was statistically significantly difference between PTV and dengue. CI = Confidence Interval; DENV = Dengue virus; PTV = Punta Toro species complex; Missing data: Age (PTV = 1, DENV = 6); Sex (DENV = 5).

†Calculated using unconditional logistic regression method. The estimates are calculated using maximum likelihood method except for fever, hepatomegaly, and splenomegaly where Firth's method was used due to zero cell frequency (2).

‡Age reported as median with quartiles.



Technical Appendix Figure. Maximum likelihood phylogenetic tree of the Punta Toro serocomplex sequences detected in dengue-negative samples of febrile patients in Panama from 2009. Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood is shown, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nt sequences of around (482 bp nucleotide long - L gene from 2096 to 2577) of which 11 were from 2009 Panamanian sequences (GMI240989 [GenBank accession no. KY435355], GMI241043 [accession no. KY435356], GMI241212 [accession no. KY435357], GMI24122 [accession no. KY435358], GMI241523 [accession no. KY435359], GMI241558 [accession no. KY435360], GMI241573 [accession no. KY435361], GMI241596 [accession no. KY435362], GMI241709 [accession no. KY435363], GMI242295 [accession no. KY435364], GMI242368 [accession no. KY435365]). The tree reliability topology was estimated using bootstrap resampling (2,000 replicates), bootstrap values are shown for each branch. Buenaventura virus was used as an outgroup. Evolutionary analyses were conducted in MEGA7 (3). From the 11 sequences, 7 are identical, whereas 2 (GMI241212 and 241222) have one mutation (bootstrap 0) and 2 (GMI240989 and 241043) have three mutations and form an inside cluster (bootstrap 88), they cluster separately from Cocle virus (11 mutations from the other 2009 Panama PTV, bootstrap 96).

References

1. Palacios G, Wiley MR, Travassos da Rosa AP, Guzman H, Quiroz E, Savji N, et al. Characterization of the Punta Toro species complex (genus *Phlebovirus*, family *Bunyaviridae*). *J Gen Virol.* 2015;96:2079–85. [PubMed http://dx.doi.org/10.1099/vir.0.000170](http://dx.doi.org/10.1099/vir.0.000170)
2. Fisher Box J. Guinness, Gosset, Fisher, and Small Samples. *Stat Sci.* 1987;1:45–52 (cited 2016 Oct 31). <http://projecteuclid.org/euclid.ss/1177013437>
3. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 2016;33:1870–4. [PubMed http://dx.doi.org/10.1093/molbev/msw054](http://dx.doi.org/10.1093/molbev/msw054)