# Serological Qualitative Diagnoses of *Helicobacter pylori* in Patients Accessing Care at the Bingham University Teaching Hospital Jos, Nigeria

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Abstract. The widespread Helicobacter pylori infection is a substantial global health problem affecting approximately 50% of the worldwide population, with 50% infection rates in developed countries and 80% in developing countries, mainly concentrating in resource-limited settings. The mode of transmission is through the faecal-oral route, contamination of food and water, where inadequate sanitation practices, low socioeconomic status and overcrowdedness seem to relate to the high prevalence of *H. pylori* infections. This study sought to serologically determine the prevalence of *H. pylori* and the disease-associated burden in patients accessing care in a Tertiary Hospital. This hospital-based cross-sectional study was conducted at the Bingham University Teaching Hospital, North-Central Nigeria, for four months (September to December 2022). There, 551 blood specimens were collected from the patients into plain tubes and spun to obtain serum for the serological qualitative analysis. Out of the 551 screened specimens for H. pylori, 79% (n=437) were 58.4% reactive female and 41.6% male. Ages 15-49 had 62%, 50-70 yrs had 26.5%, and less than 14 yrs had 11.4% respectively. Furthermore, 64.3% of female patients presented with burning pains, nausea/vomiting, and trouble breathing.

In comparison, 35.5% of the male counterparts presented symptoms of dyspepsia, and 32.1% had either taken one of the Nonsteroidal-inflammatory drugs. 86.7% of the suspected patients were hand washed after using the toilet, 83.3% had a loss of appetite, 55.4% reported alcohol intake and 35.9% smoked instead. 47.7% ate from mama-put, 30.1% from street-vended foods and 22.2% from classified restaurants, while 94.7% got their drinking water from sachet, bottled, borehole or tap, and well water, respectively.

Serum antibody detection of *H. pylori* infection was higher in female than male patients accessing care at the Bingham University Teaching Hospital, Jos. This revealed that gender could be considered a potential risk factor. Thus, early risk identification factors, such as other transmission routes, are urgently needed in defining clinical and epidemiological characteristics to facilitate appropriate supportive care and prompt treatment.

Keywords: peptic ulcer; H. pylori; seropositivity; gastric cancer; test-and-treat.

#### INTRODUCTION

Despite a recent decline in incidence, gastric ulcer, commonly known as peptic ulcer, remains one of the leading causes of gastric cancer death worldwide [1–3]. Helicobacter pylori (*H. pylori*), a gram-negative bacterium, is responsible for colonisation in a human gastric micro niche with

more than 50–84% prevalence worldwide, causing progressive genetic damage to the gastric epithelium that may eventually lead to gastric adenocarcinoma [4-7]. Over two decades, early studies demonstrated that *H. pylori* infection contributes to the development of several digestive diseases, such as dyspepsia [8, 9], peptic ulcer disease (PUD) [9, 10], and gastric cancer [11]. How-

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ever, current clinical recommendations state that H. pylori test-and-treat should be individualised based on comorbidities and patient preferences among populations at increased risk for specific morbidities because, in recent times, researchers believed that people could transmit H. pylori from person to person, especially during childhood [12]. Peptic ulcer formation depends on the presence of gastric juice pH and the decrease in mucosal defences [14]. Though *H. pylori* remain present between the mucous layer and the gastric epithelium strategically designed to reside within the aggressive environment in the antrum and migrates towards the proximal segment of the stomach, thus, for most people, the presence of *H. pylori* does not have a negative impact only 10 to 15% may end up developing ulcers [13, 141.

In the developed world, the prevalence rates of *H. pylori* vary from 1.2 to 12.2%, and the USA recorded 82%. In contrast, the rates are much higher in developing countries, with about 70% to 90% of the population harbouring *H. pylori* [15]. In Nigeria, the prevalence rate of *H. pylori* accounted for 91%, with 82% found in children between the ages of 5-9 years old [16, 17]. Research conducted in other parts of Nigeria reported the prevalence of *H. pylori* among patients attending GIT clinics to be 52% in Anambra [18], 81% in Kano [19], 77.1% in Gombe [20], 73% in Ile-Ife [21], 87% in Jos [22], 84% in Maiduguri [23].

Individuals infected with *H. pylori* develop serum antibodies which correlate strongly with histologically confirmed cases, thus, necessitating the need for the development of one step screening test kit device that utilises a combination of antigen-coated particles with antihuman immunoglobulin G (IgG) to detect H. pylori antibodies qualitatively and selectively in human blood specimen in just a few minutes. Sero-positivity indicates active or past infection both in symptoms and asymptomatic patients, which may persist for months after spontaneous or therapeutic resolution of active disease and complications increases, thereby raising the problem of growing antimicrobial resistance by reducing the efficiency of eradication therapy [15, 24 25].

In most cases, burning pain is observed in the middle or upper stomach between meals or at night, followed by discomfort that temporarily disappears if one eats something or takes an antacid. Infected patients may also experience bloating, heartburn, nausea or vomiting, dark or black

stool due to bleeding, unexplained weight loss, trouble breathing, and fainting [26].

Furthermore, the prevalence of *H. pylori* appears to affect all age groups, particularly children, where most diagnostic procedures rely on rapid urease test, culture and direct gram stain, histology, cytology, and polymerase chain reaction (PCR). However, there is an urgent need to serologically confirm and thus enable the assessment of the prevalence of *H. pylori* and the disease-associated burden in resource-limiting settings among patients accessing care this study aimed to seek.

#### **MATERIALS AND METHODS**

This hospital-based cross-sectional study was conducted at the Bingham University Teaching Hospital, North-Central Nigeria, for four months (September to December 2022). This study's sample size was determined by taking a minimum sample size from the study conducted by [22], who reported an 87% prevalence of *H. pylori* in Jos using the Cochran formula recommended by [27].

A whole blood sample of about 5 ml was collected from 551 patients suspected of peptic ulcer into a plain tube container and spun for 5 minutes in the centrifuge at 3000 RPM to obtain serum and tested to detect antibody for *H. pylori* infection using a Royal Care diagnostic kit (ISO:9001 certified) following manufacturer instructions, the samples were measured in the test cassette and the mixture determined by capillary effect. The displayed results were then recorded.

The patients' biodata, including age, sex, symptom of dyspepsia, history of smoking, alcohol intake and NSAID, was documented.

Data Analysis. Data were entered into Excel and Microsoft Word and imported into statistical packages for social science (SPSS version 26) for analyse. Frequency and percentage rate were computed for categorical variables, and the prevalence was expressed in percentage. The proportion was compared for the degree of fitness and association by the chi-square test with a level of significance set at 5% (p<0.05).

## **RESULTS AND DISCUSSIONS**

Seroprevalence of H. pylori in the study area. Out of the 551 serological specimens for H. pylori,

79.3 % (n=437) were positive. Studies [18, 28] found the prevalence of *H. pylori* at 52% and 12.7%, respectively. Also, in Korea, a study by [29] reported that the seroprevalence of *H. pylori* infection was 41.5%. This indicates that the seroprevalence of *H. pylori* in most parts of the world is significantly high. The prevalence ranges between 85% and 95% in developing countries and between 30 and 50% in developed countries, for which our study falls in the range.

In Table 1, results revealed that the female patients had higher positivity rates of 58.4% and males with 41.6%.

This showed that female folks have high intensity of the disease compared to males, which is proportionate to [18], who reported that female patients have the highest (53%) rates of *H. pylori* than the male (47%) in Anambara state with no significant difference (p=0.232). This also concurs with the report [30], which revealed that the prevalence of *H. pylori* infection was found to be

significantly higher (42.6%) among female participants than among their male (8.2%) counterparts.

Table 1 – Seroprevalence of *H. pylori* in patients accessing care at the Bingham University Teaching Hospital

Hospital				
Gender	Positive	Negative	Total (%)	P-
	(%)	(%)	10tai (%)	value
Male	182	47	229	
	(41.6)	(41.2)	(41.6)	
Female	255	67	322	
	(58.4)	(58.8)	(58.4)	
Total	437	114	551	0.9354
	(79.3)	(20.7)	(100)	0.9354

Following age groups, ages 15-49 had 62.0%, followed by 50-70 years and above with 26.5%, respectively, as seen in Figure 1.

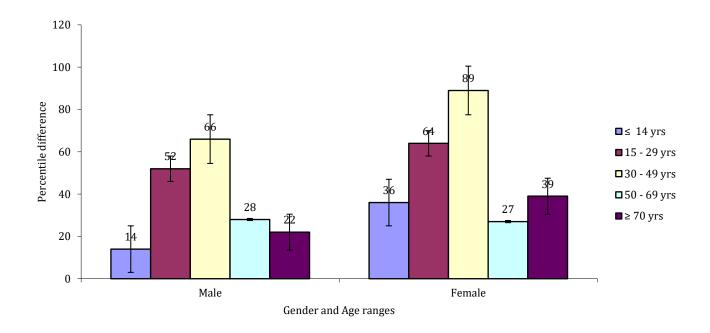


Figure 1 – Prevalence based on gender and age group

The prevalence among ages less than 14 years showed a lesser (11.4%) seropositivity rate in the study area, where studies in other parts of the developing countries reported higher rates of 82% (Nigeria), 48% (Ethiopia), 95% (Gambia), 50% (Egypt), 58% (Bangladesh), 41% (China), 22% (India), 30% (Siberia), and 52% (Peru) in ages between 0-12 years old. Authors [31] reported that the prevalence of *H. pylori* infection

was 18.2% among 6–59 months old children, 14% among adolescent boys and 16% among teenage girls aged 10–19 years, and 40% among 20–49 years non-pregnant women.

*Symptoms of dyspepsia*. For the discomfort or pain that occurs in the upper abdomen in the patients, our study revealed that 64.3% of the female patients had reported feeling burning pains, nausea or vomiting and having trouble breathing.

In comparison, 35.5% of the male counterparts experienced symptoms of dyspepsia. Though in Nigeria, various studies on *H. pylori* confirmed high prevalence rates between 73.0% and 94.5% among patients with dyspepsia [12]. Those with pain, including discomfort in the upper abdomen and symptoms resembling those of ulcer and had taken any pain reliever or drug were 32.1%. However, this study discovered that symptoms don't always happen, but functional dyspepsia is common among them, as seen in Table 2 below.

Table 2 – Symptoms of Dyspepsia in patients suspected to have a peptic ulcer in the study area

Variables	Male (%)	Female (%)	Total	P-value
Feel burning pain	83 (38.6)	132 (61.4)	215	
Feel nauseous or vomiting	68 (29.8)	160 (70.2)	228	
Have trouble breathing	39 (38.2)	63 (61.2)	102	
Any pain reliever or drug for the pain	79 (44.6)	98 (55.4)	177	
If yes, which one				
Antacid/MMT	59 (41.3)	84 (58.7)	143	
Ibuprofen	43 (39.8)	65 (60.2)	108	
Aspirin	18 (60.0)	12 (40.0)	30	
Misoprostol	21 (21.9)	75 (78.1)	96	
Omeprazole	56 (40.0)	84 (60.0)	140	0.0014*

Notes: \*Significant p-value < 0.05

Risk factors assessment. Table 3 revealed that most (86.7%) suspected patients were hand washed after using the toilet to prevent infection. This is agreed with the continued Epidemiologic studies to demonstrate the favourable costbenefit ratio and positive effects of simple hand flowing for preventing transmission of pathogens in healthcare facilities [32]. Many (83.3%) lose their appetite, followed by alcohol intake (55.4%), and 35.9% for smoking instead. Regarding sources of food, normally eating away from home, 47.7% of them eat from mama-put, 30.1% for street-vended foods and 22.2% for classified restaurants. Many (94.7%) of the patients got their source of drinking water mainly from sa-

chet, bottled, borehole or tap, and well water, respectively.

Table 3 – Dyspepsia risk factors amongst patients seen in the study area

Seen in the study area							
Variables	Yes	No	Total	P-value			
	(%)	(%)	(%)				
Do you smoke	157	280	427				
-	(35.9)	(64.1)	437				
Alcohol intake	242	195	127				
	(55.4)	(44.6)	437				
loss of appetite	364	73	437				
	(83.3)	(16.7)	437				
hand washing after	379	58	437				
using the toilet	(86.7)	(13.3)					
Source of food norm	ally eati	ng away	from				
home.	_						
Mama put	103	127	230				
(unclassified	(47.7)	(57.5)	(52.6)				
restaurants)	,		,				
Street food	65	69	134				
(vendors)	(30.1)	(31.2)	(30.7)				
Restaurants	48	25	73				
(classified)	(22.2)	(11.3)	(16.7)				
Total	216	221	437	0.0073*			
	(49.4)	(50.6)	(100)	0.0073			
Source of drinking v							
Sachet water	194	9	203				
	(95.6)	(4.4)	203				
Bottled water	74	4	78				
	(94.8)	(5.1)	70				
Well water	58	6	64				
	(90.6)	(9.4)	04				
Pipe or tap water	129	8	137				
	(94.2)	(5.8)	137				
Borehole water	67	2	69				
	(97.1)	(2.9)					
Total	522	29	551	0.5061			
	(94.7)	(5.3)	(100)	0.5001			

Notes: \*Significant p-value < 0.05

#### **CONCLUSIONS**

Serological qualitative serum antibody detection of *H. pylori* infection was higher in female than male patients accessing care at the Bingham University Teaching Hospital, Jos. This revealed that gender could be a potential risk factor for *H. pylori*. Thus, early risk identification factors for *H. pylori* because of food and water contamination are urgently needed in defining clinical and epidemiological characteristics with greater precision to facilitate appropriate supportive care and prompt treatment.

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### **Conflict of interest**

The authors declared no conflicting interests whatsoever that could lead to bias.

#### **REFERENCES**

- 1. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424. doi: 10.3322/caac.21492
- 2. Liou, J.-M., Malfertheiner, P., Lee, Y.-C., Sheu, B.-S., Sugano, K., Cheng, H.-C., Yeoh, K.-G., Hsu, P.-I., Goh, K.-L., Mahachai, V., Gotoda, T., Chang, W.-L., Chen, M.-J., Chiang, T.-H., Chen, C.-C., Wu, C.-Y., Leow, A. H.-R., Wu, J.-Y., Wu, D.-C., ... El-Omar, E. M. (2020). Screening and eradication of Helicobacter pylori for gastric cancer prevention: the Taipei global consensus. *Gut*, *69*(12), 2093–2112. doi: 10.1136/gutjnl-2020-322368
- 3. Talebi Bezmin Abadi, A. (2018). Diagnosis of Helicobacter pylori Using Invasive and Noninvasive Approaches. *Journal of Pathogens, 2018,* 1–13. doi: 10.1155/2018/9064952
- 4. Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., Malfertheiner, P., Graham, D. Y., Wong, V. W. S., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., Kaplan, G. G., & Ng, S. C. (2017). Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology*, 153(2), 420–429. doi: 10.1053/j.gastro.2017.04.022
- 5. Melese, A., Genet, C., Zeleke, B., & Andualem, T. (2019). Helicobacter pylori infections in Ethiopia; prevalence and associated factors: a systematic review and meta-analysis. *BMC Gastroenterology*, 19(1). doi: 10.1186/s12876-018-0927-3
- 6. Venneman, K., Huybrechts, I., Gunter, M. J., Vandendaele, L., Herrero, R., & Van Herck, K. (2018). The epidemiology of Helicobacter pylori infection in Europe and the impact of lifestyle on its natural evolution toward stomach cancer after infection: A systematic review. *Helicobacter*, *23*(3), e12483. doi: 10.1111/hel.12483
- 7. Zamani, M., Ebrahimtabar, F., Zamani, V., Miller, W. H., Alizadeh-Navaei, R., Shokri-Shirvani, J., & Derakhshan, M. H. (2018). Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. *Alimentary Pharmacology Therapeutics*, *47*(7), 868–876. doi: 10.1111/apt.14561
- 8. Li, L., Tan, J., Liu, L., Li, J., Chen, G., Chen, M., Xie, J., Song, Q., Huang, X., & Xie, S. (2020). Association between *H. pylori* infection and health Outcomes: an umbrella review of systematic reviews and meta-analyses. *BMJ Open, 10*(1), e031951. doi: 10.1136/bmjopen-2019-031951
- 9. Eslick, G. D., Lim, L. L.-Y., Byles, J. E., Xia, H. H.-X., & Talley, N. J. (1999). Association of Helicobacter Pylori Infection With Gastric Carcinoma: A Meta-Analysis. *American Journal of Gastroenterology*, 94(9), 2373–2379. doi: 10.1111/j.1572-0241.1999.01360.x
- 10. Vergara, M., Calvet, X., & Roqué, M. (2002). Helicobacter pylori is a risk factor for peptic ulcer disease in cirrhotic patients. A meta-analysis. *European Journal of Gastroenterology Hepatology,* 14(7), 717–722. doi: 10.1097/00042737-200207000-00002
- 11. Jaiswal, F., Rai, A. K., Wal, P., Wal, A., & Singh, S. P. (2021). Peptic Ulcer: A Review On Etiology, Pathogenesis And Treatment. *Asian Journal of Pharmaceutical Education and Research*, *10*(4), 1. doi: 10.38164/ajper/10.4.2021.1-17
- 12. Jaakkimainen, R. L., Boyle, E., & Tudiver, F. (1999). Is Helicobacter pylori associated with non-ulcer dyspepsia and will eradication improve symptoms? A meta-analysis. *BMJ*, *319*(7216), 1040–1044. doi: 10.1136/bmj.319.7216.1040

- 13. Olokoba, A. B., Gashau, W., Bwala, S., Adamu, A., & Salawu, F. K. (2013). Helicobacter pylori infection in Nigerians with dyspepsia. *Ghana medical journal*, *47*(2), 79–81.
- 14. Driscoll, L. J., Brown, H. E., Harris, R. B., & Oren, E. (2017). Population Knowledge, Attitude, and Practice Regarding Helicobacter pylori Transmission and Outcomes: A Literature Review. *Frontiers in Public Health, 5.* doi: 10.3389/fpubh.2017.00144
- 15. Chey, W. D., Leontiadis, G. I., Howden, C. W., & Moss, S. F. (2017). ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. *American Journal of Gastroenterology, 112*(2), 212–239. doi: 10.1038/ajg.2016.563
- Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A., & Smolic, M. (2019). Peptic Ulcer Disease: A Brief Review of Conventional Therapy and Herbal Treatment Options. *Journal of Clinical Medicine*, 8(2), 179. doi: 10.3390/jcm8020179
- 17. Mungazi, S. G., Chihaka, O. B., & Muguti, G. I. (2018). Prevalence of Helicobacter pylori in asymptomatic patients at surgical outpatient department: Harare hospitals. *Annals of Medicine and Surgery*, *35*, 153–157. doi: 10.1016/j.amsu.2018.09.040
- 18. Pilotto, A., Di Mario, F., Franceschi, M., Leandro, G., Soffiati, G., Scagnelli, M., Bozzola, L., & Valerio, G. (1996). Cure of Helicobacter pylori infection in the elderly: effects of eradication on gastritis and serological markers. *Alimentary pharmacology & therapeutics, 10*(6), 1021–1027. doi: 10.1046/j.1365-2036.1996.88260000.x
- 19. Gisbert, J. P. (2012). Rescue Therapy forHelicobacter pyloriInfection 2012. *Gastroenterology Research and Practice*, 2012, 1–12. doi: 10.1155/2012/974594
- 20. Nwachukwu, E. P., Onwurah, O. W., Amilo, G. I., Onwuasoanya, U. F., Ezeugwunne, I. P. (2020). Prevalence of Helicobacter pylori among Patients with Gastritis Attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. *Annals of Current Gastroenterology Reports*, *1*(1), 1–4.
- 21. Tijjani, B., & Umar, A. (2009). Peptic ulcer disease and Helicobacter pylori infection at Kano, Nigeria. *The Internet Journal of Gastroenterology*, 8(1), 1–3.
- 22. Mustapha, S., Pindiga, U., Yusuph, H., Goni, B., & Jibrin Y. (2011). Helicobacter Pylori Infection Among Dyspeptic Patients at a Tertiary Hospital in Northern Nigeria. *The Internet Journal of Infectious Diseases*, 9(2), 1–4.
- 23. Ndububa, D. A., Agbakwuru, A. E., Adebayo, R. A., Olasode, B. J., Olaomi, O. O., Adeosun, O. A., & Arigbabu, A. O. (2001). Upper gastrointestinal findings and incidence of Helicobacter pylori infection among Nigerian patients with dyspepsia. *West African journal of medicine*, *20*(2), 140–145.
- 24. Malu, A. O., Ani, A. E., & Bello, S. S. (2000) The prevalence of *Helicobacter pylori* in dyspeptic patients from the Jos Plateau, Nigeria. *Nigerian Medical Journal*, 41(9), 1–3.
- 25. Holcombe, C., Umar, H., Lucas, S. B., & Kaluba, J. (1994). Low incidence of clinically significant gastroduodenal pathology despite a high incidence of Helicobacter pylori infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 88*(5), 569–571. doi: 10.1016/0035-9203(94)90166-x
- 26. Lanas, A. (2016). We Are Using Too Many PPIs, and We Need to Stop: A European Perspective. American Journal of Gastroenterology, 111(8), 1085–1086. doi: 10.1038/ajg.2016.166
- 27. Choi, I. J. (2013). Current evidence of effects of Helicobacter pylori eradication on prevention of gastric cancer. *The Korean Journal of Internal Medicine, 28*(5), 525. doi: 10.3904/kjim.2013.28.5.525
- 28. Dotsenko, S., Samura, B., Sychov, R., Tokarenko, I., Kravchenko, V., & Svistun, S. (2015). *Casebook in Gastroenterology*. Retrieved from http://dspace.zsmu.edu.ua/handle/123456789/2481
- 29. Thrusfield, M. (2007). Veterinary Epidemiology (3rd ed.). London: Blackwell Science.

- 30. Jemikalajah, D., & Okogun, G. (2014). Health Point Prevalence of Helicobacter Pylori in Central Hospital Warri, Nigeria. *African Journal of Cellular Pathology*, *3*(12),57-60.
- 31. Lim, S. H., Kim, N., Kwon, J. W., Kim, S. E., Baik, G. H., Lee, J. Y., Park, K. S., Shin, J. E., Song, H. J., Myung, D.-S., Choi, S. C., Kim, H. J., Yim, J. Y., & Kim, J. S. (2018). Trends in the seroprevalence of Helicobacter pylori infection and its putative eradication rate over 18 years in Korea: A cross-sectional nationwide multicenter study. *PLOS ONE, 13*(10), e0204762. doi: 10.1371/journal.pone.0204762
- 32. Samson, E. S. (2018). Screening for Helicobacter Pylori Infection among Undergraduate Students of a Tertiary Institution using serum Antibody and Stool Antigen Detection Methods. *Biomedical Journal of Scientific & Technical Research*, 3(2). doi: 10.26717/bjstr.2018.03.000883
- 33. Mehata, S., Parajuli, K. R., Pant, N. D., Rayamajhee, B., Yadav, U. N., Mehta, R. K., Jha, P., Mehta, N., Dhimal, M., & Singh, D. R. (2021). Prevalence and correlates of Helicobacter pylori infection among under-five children, adolescent and non-pregnant women in Nepal: Further analysis of Nepal national micronutrient status survey 2016. *PLOS Neglected Tropical Diseases, 15*(6), e0009510. doi: 10.1371/journal.pntd.0009510
- 34. Pittet, D., Sax, H., Hugonnet, S., & Harbarth, S. (2004). Cost Implications of Successful Hand Hygiene Promotion. *Infection Control & Hospital Epidemiology*, *25*(3), 264–266. doi: 10.1086/502389