Mixed transmission modes promote persistence of an emerging tick-borne pathogen

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**Abstract.** Pathogens utilize different modes of transmission to maximize transmission success. In vector-borne disease systems, both vertical and horizontal modes of transmission are common, but the relative contribution of these modes is not well understood but may be determined by host genetics, physiology, or environmental conditions. This study focuses on an emerging tick-borne relapsing fever pathogen, *Borrelia miyamotoi*, that can be transmitted both vertically and horizontally. The enzootic cycle of this pathogen has not been described in the western USA where it was recently found in the tick species, *Ixodes pacificus*. Our field surveys found that all three life stages of *I. pacificus* carry the pathogen, and therefore, all stages pose some level of disease risk to humans. The prevalence of infection increases with each life stage suggesting that horizontal transmission is important in the persistence of this pathogen in the enzootic cycle. In support of this finding, we found that small mammal hosts that are frequently parasitized by juvenile stages of *I. pacificus* were infected with *B. miyamotoi* and may therefore function as a source of horizontal transmission and enzootic maintenance of this disease. Our data show that in the western USA *B. miyamotoi* is maintained in natural populations by both transovarial transmission and transmission from blood meal hosts and that synchronous phenology of juvenile stages of *I. pacificus* may facilitate the transmission dynamics of *B. miyamotoi* and other vertically transmitted, vector-borne pathogens.

**Key words:** *Borrelia*; epidemiology; *Ixodes*; reservoir; tick; vector; vector-borne disease; vertical transmission; zoonoses.

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**INTRODUCTION**

Pathogens can utilize multiple transmission modes for maintenance within a population. While many of the most widespread vector-borne zoonotic pathogens are horizontally transmitted (Kjos et al. 2009, Reese et al. 2010, Moyes et al. 2014), vertical transmission, also known as transovarial transmission, is another possible transmission mode that has received less attention (Fine 1975). Persistence of vertically transmitted pathogens is not well understood and often assumed to occur solely through vertical transmission because the pathogen can be transmitted from one generation to the next (Ewald 1987, Bull et al. 1991, Frank 1997). In this study, we test this assumption by examining a vertically transmitted, emerging vector-borne disease to determine the relative contribution of horizontal transmission to pathogen persistence. Some vertically transmitted pathogens can be maintained entirely through vertical transmission,
such as the nearly ubiquitous insect pathogen, *Wolbachia* (McGraw and O’Neill 1999). Many tick endosymbionts are also believed to be maintained purely through vertical transmission (Noda et al. 1997). But most vertically transmitted pathogens exhibit both vertical transmission and horizontal transmission such as *Rickettsia rickettsia* in *Dermacentor andersoni* ticks, Zika virus in *Aedes aegypti* mosquitoes, and the tick-borne relapsing fever bacterium, *Borrelia miyamotoi* (Burgdorfer and Varma 1967, Scoles et al. 2001, Thangamani et al. 2016). However, the relative importance of horizontal transmission in these vertically transmitted pathogen systems has rarely been experimentally evaluated.

In North America and Europe, *Ixodes* spp. ticks transmit the most prevalent vector-borne disease—the horizontally transmitted pathogen, Lyme disease, caused by genospecies within the *Borrelia burgdorferi* sensu lato complex. *Ixodes* ticks also transmit an emerging relapsing fever pathogen, *B. miyamotoi*, which is vertically transmitted from infected adult females to her progeny. While the abundance and reservoir competency of blood meal hosts directly impact Lyme disease transmission and public health risk (LoGiudice et al. 2003), it is not known if host composition has any impact on the risk of *B. miyamotoi*. Because *B. miyamotoi* was only determined to be a zoonotic agent in 2011 (Platney et al. 2011), there is still much to learn about the enzootic cycle of this pathogen. In the eastern USA, *B. miyamotoi* is maintained by *Ixodes scapularis* and the white-footed mouse, *Peromyscus leucopus* (Barbour et al. 2009). But in the western USA, where *B. miyamotoi* is transmitted by *I. pacificus*, the role of horizontal transmission from blood meal hosts is uncertain. Serological studies have found high prevalence of *B. miyamotoi* in human populations with high exposure rates to *I. pacificus* (Krause et al. 2018), and there is a strong potential for it to emerge as a zoonotic pathogen in this region given genetic similarity to strains isolated from the eastern USA where clinical cases have been confirmed (Crowder et al. 2014, Molloy et al. 2015, Wroblewski et al. 2017). Recent comparative experimental work determined that *I. scapularis* and *I. pacificus* acquire *B. burgdorferi* from blood meal hosts at similar levels of efficiency especially when using sympatric bacterial strains that co-evolved with the vector species (Couper et al. 2020). These findings suggest that the *I. scapularis* and *I. pacificus* are equally competent vector species for *B. burgdorferi*, and potentially *B. miyamotoi* as well.

The relative importance of horizontal transmission could have important implications for predicting and managing the risk of disease (Cutler 2015, Lynn et al. 2018). For instance, if horizontal transmission is the key to persistence of a vertically transmitted pathogen, then disease risk can be predicted based on host composition and can be managed by targeting hosts for treatment, rather than the vector (Tsao et al. 2004, Barbour et al. 2009). Here, we examine *B. miyamotoi* infection prevalence in *I. pacificus* life stages and blood meal hosts to determine the mechanisms of persistence of this emerging pathogen.

**Materials and Methods**

Site selection and set up

Eight sites in oak woodland habitat were sampled from April to May in 2018 in northern California (CA). Four of these sites had previous published reports of *B. miyamotoi* prevalence in *I. pacificus* (Padgett et al. 2014, Salkeld et al. 2015): Windy Hill Open Space Preserve (Windy Hill; 37°21’52.0”, –122°13’13”), Filoli Estates (Filoli; 37°28’04.4”, –122°18’48.2”), and Junipero Serra County Park (Junipero Serra; 37°36’28.5”, –122°25’26.8”) in San Mateo County, CA; China Camp State Park (China Camp), Marin County, CA (38°00’04.7”, –122°29’19.8”). Four of the sites had no previous surveillance for *B. miyamotoi* but are habitats with known populations of *I. pacificus* ticks: Lafayette Reservoir (Lafayette; 37°53’02.2”, –122°08’05.1”) in Contra Costa County, CA; Spring Lake Regional Park (Spring Lake; 38°27’08.1”, –122°38’54.2”) in Sonoma County, CA; and Heinz Open Space (Heinz; 37°13’38.3”, –121°55’24.6”) and Worcester Park (Worcester; 37°13’17.4”, –121°57’58.0”) in Santa Clara County, CA (Lawrence et al. 2018). Each site was sampled on a half-hectare grid using a 7 × 7 trapping array with 11.8 m spacing between trapping stations and 49 sampling stations. Each grid and trapping array were standardized following Lawrence et al. (2018).
Collection methods

Host-seeking I. pacificus ticks were collected using standard dragging method with a 1-m² white flannel cloth totaling a sampling area of 495 m² at each site. Drag cloths were checked every 12 m, and collected ticks were stored in 70% ethanol (EtOH) until identification and molecular testing (Mtambo et al. 2006). Tick drag sampling occurred twice at each site, once during small mammal trapping in April 2018 and once at the end of May 2018. Three of the eight sites (Windy Hill, Filoli, and China Camp) were sampled a third time to target peak abundance of adult ticks in February.

Small mammal trapping was conducted for three consecutive days at each site. One site, Windy Hill, was sampled on a second occasion a month later. Extra-long Sherman traps (7.6 x 9.5 x 30.5 cm; H.B. Sherman Traps, Tallahassee, Florida, USA) were used to sample small mammals. Two traps were placed at each of the 49 trapping stations, for a total of 98 traps per site per night. Each trap was baited with oats mixed with peanut butter and cotton for cold (<10°C) nights. Traps were set in the afternoon and checked early morning. Each animal was given a unique ear tag number (National Band and Tag, Newport, Kentucky, USA) and identified to species. For each animal caught, data were recorded on body mass, sex, and reproductive status. From each animal, we collected all attached ticks, up to 150 µL of whole blood, and 2-mm ear biopsies. During blood collection, animals were anesthetized with a cotton ball containing 5 mL of 50% isoflurane/propanediol glycol solution in a bag. Animals did not come into direct contact with the isoflurane solution and were monitored during the procedure. Whole blood was collected in non-heparin capillary tubes via retro-orbital bleeding and stored in EDTA tubes on ice until frozen in liquid nitrogen and stored at ~80°C until analysis. Ear biopsies were stored in 70% EtOH at 4°C until nucleic acid extraction.

All animal handling protocols were approved by the institutional animal care and use committee at San Francisco State University (#AU16-05R2b).

Lab analysis

Collected ticks were identified to life stage and species using taxonomic keys (Furman and Loomis 1984). DNA was extracted from mammal whole blood samples, ear tissue samples, and individual ticks with a DNeasy blood and tissue kit (Qiagen, Valencia, California, USA). Extracted DNA was stored at −20°C. Pathogen testing utilized a nested polymerase chain reaction (PCR) targeting the 16S-23S rDNA intergenic spacer (IGS) that simultaneously amplifies B. miyamotoi and B. burgdorferi (Bunikis et al. 2004, Ullmann et al. 2006). A second nested PCR targeting the 16S rDNA gene (Fomenko et al. 2011) was used to identify an uncharacterized Borrelia sp. that clustered with relapsing fever group spirochetes using the 16S-23S rDNA IGS (Scoles et al. 2001). All samples were tested by nested PCR in triplicate with negative and positive controls. All collected blood samples were tested. Ear punch tissue from an individual animal was only tested if a site had positive B. miyamotoi blood samples because of the higher detection success of B. miyamotoi in blood (Barbour et al. 2009). Not all animals tested had both blood and tissue sample collected due to time constraints in the field. PCR products were visualized in a 1.8% agarose gel stained with ethidium bromide. Purification of PCR amplicons used SeraPure magnetic beads and amplicons were sequenced on an Applied Biosystems 3730 using forward and reverse internal primers (Life Technologies, Grand Island, New York, USA). Sequences were edited in Geneious Prime 2019.2.3 (https://www.geneious.com) and aligned to reference sequences using BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi). In addition, a PCR targeting the mammal cytb gene (Smith and Patton 1991) was performed on all positive animals to confirm species identification of Peromyscus spp. due to regional variation in morphological traits. A phylogenetic tree was generated to determine the relationship of B. miyamotoi, the novel Borrelia sp., and other borreliae. All 16S rRNA sequences were acquired from GenBank with the exception of the novel Borrelia sp. from this study. A phylogenetic tree was constructed in Geneious Prime 2019.2.3 (https://www.geneious.com) using Geneious Alignment with 70% similarity. A tree was built using PhyML with the substitution model Jukes-Cantor (JC69) with bootstrap values after 500 simulations.
RESULTS

Small mammal infection

A total of 370 small mammals were captured from eight sites between April and May 2018 over 2304 trap nights. We collected 248 blood samples and 236 tissue samples from seven mammal species. Both blood and tissue were collected from 180 animals (Appendix S1: Table S1). We detected *B. miyamotoi* in rodents from five of the eight sites examined in this study (Fig. 1). The prevalence of *B. miyamotoi* in rodent blood samples across the five positive sites ranged from 2.0% to 7.41%. One site, Windy Hill, had the highest *B. miyamotoi* rodent blood prevalence and was sampled twice in a five-week period with 0.0% (95% Bayesian credible interval [BCI] 0.06–8.60) prevalence in April and 19.0% (95% BCI 8.66–43.7) prevalence in May.

Small mammal trapping resulted in samples from seven rodent species: deer mouse (*Peromyscus maniculatus*), California mouse (*Peromyscus californicus*), piñon mouse (*Peromyscus truei*), dusky-footed woodrat (*Neotoma fuscipes*), California vole (*Microtus californicus*), and western harvest mouse (*Reithrodontomy megalotis*). Tomahawk traps were used to capture *Sciurus* spp. (*S. griseus*, *S. carolinensis*, *S. niger*). *Borrelia miyamotoi* was detected in blood samples from *P. truei*, *P. maniculatus*, and *P. californicus*, with the highest prevalence found in *P. truei* (6.02%; 95% BCI 3.16–11.59%; Table 1). The prevalence of *B. miyamotoi* in *P. californicus* was 3.85% (95% BCI 0.98–20.35%) and *P. maniculatus* was 2.86% (95% BCI 0.72–15.33%), while all other species tested (*N. fuscipes*, *Sciurus* spp., *M. californicus*, *R. megalotis*) were negative for *B. miyamotoi* (Table 1). One of the positive *P. truei* caught at Windy Hill during the second trapping session was negative for *B. miyamotoi* in April but positive in May.

We only detected *B. miyamotoi* in blood samples of *Peromyscus* species and did not detect the pathogen in ear tissue. In contrast, *B. burgdorferi* sensu stricto (s.s.) was more likely to be detected in ear tissues than blood. We detected *B. burgdorferi* s.s. in *P. truei* and *P. maniculatus* at prevalences of 3.70% (2/154) and 8.33% (1/12). We also detected *Borrelia bissettiae*, a genomospecies in the *B. burgdorferi* sensu lato group, in rodent tissue at a prevalence of 3.81% (95% BCI of 2.06–7.15%). Eight of the *B. bissettiae* infections were detected in *N. fuscipes* and two in *P. truei* (Appendix S1: Table S2). An uncharacterized relapsing fever *Borrelia* species was detected at a prevalence of 14.1% (95% BCI 10.4–19.1%) in *Peromyscus* blood samples and was predominantly found in *P. truei* (Appendix S1: Table S2). Two *P. truei* blood samples were coinfected with two different *Borrelia* species. One sample was...
co-infected with *B. miyamotoi* and the uncharacterized relapsing fever *Borrelia* species, while a second sample was co-infected with *B. burgdorferi* and the uncharacterized *Borrelia* species. This uncharacterized species had 96.8% 16S rRNA (*E* value: 0.0) and 89.9% 16S-23S rDNA IGS (*E* value: 2e-54) sequence similarity to other tick-borne relapsing fever spirochetes but did not have an exact match in the NCBI database. The GenBank accession numbers for all positive samples from this study are as follows: MN110040–MN110109 (Appendix S1: Table S3).

**Questing tick infection**

Across all sampled sites, we collected 1358 questing *I. pacificus* ticks that were composed of 894 larvae, 281 nymphs, and 183 adults (Table 2). The infection prevalence of *B. miyamotoi* in *I. pacificus* increased from the larval to nymph to adult life stages (Fig. 2). The nymphal infection prevalence was more than 45-fold greater than the larval infection prevalence, and the adult infection prevalence was 1.8-fold greater than the nymphal infection prevalence. Meanwhile, the infection prevalence of *B. burgdorferi* s.s. in *I. pacificus* decreased from the nymph to adult stage, 9.86% (28/284) to 2.33% (2/86), a 76.4% decrease. Two *I. pacificus* nymphs were infected with both *B. miyamotoi* and *B. burgdorferi*.

Tick samples positive for *B. miyamotoi* by 16S-23S rRNA IGS PCR were confirmed to be *B. miyamotoi* by sequencing. However, we were not able to produce readable sequence from one sample. *Borrelia burgdorferi* sequences were also confirmed using sequences from the 16S-23S rRNA IGS locus (Appendix S1: Table S3).

Phylogenetic analysis based on 16S rRNA sequencing showed that the novel *Borrelia* sp. is distinct from the *B. burgdorferi* sensu lato Lyme disease group (*Borrelia afzelii*, *Borrelia garinii*, *B. burgdorferi*, and *Borrelia bissettii*) and clusters with relapsing fever spirochetes (*Borrelia hermsii*, *Borrelia parkeri*, *B. miyamotoi*, *Borrelia lonestari*) but not most closely related to *B. miyamotoi* (Fig. 3).

**DISCUSSION**

We show that all life stages of *I. pacificus* harbored *B. miyamotoi* and the mean infection prevalence increased through the tick’s ontogenic development from larval to nymph to adult life stages. These results strongly suggest that in addition to transovarial transmission, *I. pacificus* also can be infected with *B. miyamotoi* over the course of its life stage development, most likely acquiring the pathogen from blood meal sources. Our analysis of tick blood meal hosts supports...
these results as *Peromyscus* spp. mice were infected with *B. miyamotoi* at a prevalence of 5.21%. Together, these findings suggest that blood meal hosts contribute to the maintenance and enzootic transmission of this emerging relapsing fever pathogen in the far-western USA.

As a vertically transmitted pathogen, it is possible that *B. miyamotoi* can be maintained without a reservoir host. However, we found that infection prevalence of questing larvae was very low, only 0.11%. In contrast, questing nymphs that have had one blood meal, and therefore one opportunity for horizontal transmission, had a significantly higher infection prevalence with *B. miyamotoi* of 5.0%, a value that is more than 45 times greater than the larval infection prevalence (Table 2). Based on our measured infection prevalence in larvae, we estimate that horizontal transmission accounts for >97% of the *B. miyamotoi* infections in nymphal ticks, and 43% of the infections in adult ticks. We conclude that horizontal transmission from blood meal hosts contributes significantly to the infection prevalence of *B. miyamotoi* in *I. pacificus* and amplifies the potential for zoonotic disease transmission from nymphal ticks. While larvae can be infected with *B. miyamotoi* and therefore pose some disease risk, especially because of their miniscule size, their infection prevalence with *B. miyamotoi* is quite low. Nymph and adult *I. pacificus*, on the other hand, have significantly higher infection prevalence and therefore pose a higher zoonotic

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**Table 2. *Borrelia miyamotoi* prevalence by site in *Peromyscus* spp. blood samples and *Ixodes pacificus* ticks with 95% Bayesian credible intervals (% BCI).**

| Site          | All *Peromyscus* species | Larvae | Nymphs | Adults |
|---------------|--------------------------|--------|--------|--------|
|               | Prevalence (%) | N    | BCI (%) | Prevalence (%) | N    | BCI (%) | Prevalence (%) | N    | BCI (%) |
| Spring Lake   | 7.14          | 14   | 1.92–36.03 | 1.56          | 64   | 0.39–8.53 | 0.00          | 2    | 2.50–97.50 |
| China Camp    | 0.00          | 27   | 0.10–13.22 | 6.19          | 97   | 2.98–13.10 | 0.00          | 14   | 0.19–24.70 |
| Junipero Serra | 2.50         | 40   | 0.63–13.47 | 0.00          | 24   | 0.11–14.80 | 0.00          | 0    | NA      |
| Lafayette     | 0.00          | 5    | 0.63–60.24 | 10.0          | 10   | 2.81–48.20 | 0.00          | 0    | NA      |
| Filoli        | 6.82          | 44   | 2.59–19.06 | 3.03          | 33   | 0.77–16.20 | 0.00          | 29   | 0.09–12.34 |
| Windy Hill    | 8.00          | 50   | 3.40–19.60 | 8.77          | 57   | 4.03–19.60 | 11.7          | 137  | 7.45–18.40 |
| Heinz         | 9.09          | 11   | 2.52–44.50 | 0.50          | 201  | 0.12–2.754 | 0.00          | 19   | 0.14–18.53 |
| Worcester     | 0.00          | 1    | 100–100    | 0.00          | 13   | 0.21–26.46 | 0.00          | 1    | 100–100  |
| Total         | 5.21          | 192  | 2.91–9.42  | 0.11          | 894  | 0.03–0.622 | 4.98          | 281  | 3.03–8.25 |

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**Fig. 2.** Prevalence of *Borrelia miyamotoi* across the three life stages of *Ixodes pacificus*. *Borrelia miyamotoi* prevalence across all *I. pacificus* life stages (larvae, nymphs, adults) at eight field sites. Error bars represent 95% Bayesian credible intervals.
risk because they are much more likely to transmit the pathogen to humans.

In our field surveys, we encountered and sampled seven mammal species but only three species, *P. truei*, *P. maniculatus*, and *P. californicus*, were infected with *B. miyamotoi*. The pinion mouse, *P. truei*, had higher mean infection prevalence with *B. miyamotoi* than *P. maniculatus* and *P. californicus*, but it is not possible to determine whether these prevalences are significantly different from each other, so it remains to be seen if relative reservoir competency differs between these deer mice species. Small mammal infection with *B. miyamotoi* was only detected from rodent blood samples, while *B. burgdorferi* was more commonly found in ear tissue. In addition, we identified an uncharacterized *Borrelia* sp. in blood samples of *Peromyscus* spp. but not ticks. Phylogenetic analysis places this *Borrelia* sp. in the same clade as other relapsing fever spirochetes such as *B. miyamotoi* and *B. burgdorferi*.

*Borrelia miyamotoi* infection in wild rodents is generally believed to be transient (Taylor et al. 2013). This is one reason why the importance of horizontal transmission from blood meal hosts is uncertain. However, the duration of *B. miyamotoi* infection in hosts has never been directly measured and warrants further exploration through longitudinal field or laboratory infection studies to determine the generalizability and duration of a transient infection in rodents. At a minimum, our detection of *B. miyamotoi* in *Peromyscus* spp. throughout the larval and nymphal questing season indicates that these mice are infected long enough for us to detect circulating pathogens in the blood, during which time they can serve as a source of horizontal transmission to feeding ticks. Due to time and other logistical constraints, we only conducted repeated sampling at one site (Windy Hill), but at this site, we found direct evidence that pathogen transmission between ticks and hosts was occurring, further supporting the idea that horizontal transmission is an important component of the *B. miyamotoi* enzootic cycle.

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**Fig. 3.** Phylogenetic tree of *Borrelia* species, including the uncharacterized *Borrelia* sp. (*Borrelia* sp. novel). All sequences were acquired from GenBank with the exception of the novel *Borrelia* sp. from this study. A phylogenetic tree was built in Geneious Prime 2019.2.3 (https://www.geneious.com) using Geneious Alignment with 70% similarity. A tree was built using PhyML with the substitution model Jukes-Cantor (JC69) with bootstrap values after 500 simulations. Sequence reference numbers for relapsing fever spirochetes: *Borrelia lonestari* (AY_682921), *Borrelia miyamotoi* (NR_025861), *Borrelia hermsii* (KX_171924), and *Borrelia parkeri* (AY_934594). Sequence reference numbers for Lyme disease spirochetes: *Borrelia afzelii* (D67019), *Borrelia burgdorferi* (NR_044732), and *Borrelia bissetti* (NR_148750), *Borrelia garinii* (NR_043413).
We found that in many sites, *I. pacificus* are more likely to be infected with *B. miyamotoi* than *B. burgdorferi*. This finding may be attributed to the physiology of *I. pacificus* life stages. In the eastern USA, *I. scapularis* infection prevalence increases with each life stage (larvae, nymph, adult) because nymphs emerge earlier in the season than larvae and can infect blood meal hosts (Kurtenbach et al. 2006, Ostfeld et al. 2006, Levi et al. 2015). However, in the western USA, larval and nymphal *I. pacificus* activity are more synchronous (Padgett and Lane 2001, MacDonald and Briggs 2016), leading to less efficient transmission of *B. burgdorferi* from nymphs to larvae (Kurtenbach et al. 2006). However, more synchronous juvenile tick activity may benefit transmission of a vertically transmitted pathogen, particularly one with a putatively short-lived infection in the host. The phenology of juvenile stages of *I. pacificus* allows infected larvae to transmit *B. miyamotoi* to the nymphal cohort through transient infection in their shared blood meal hosts (i.e., *Peromyscus* mice). Thus, the human risk of *B. miyamotoi* in the western USA may be even greater than *B. burgdorferi* in certain disease hot spots as reported here and elsewhere (Crowder et al. 2014).

Emerging infectious diseases are an increasing threat to humans and animals on a global scale (Woolhouse and Gowtage-Sequeria 2005, Jones et al. 2008). Vector-borne diseases comprise of a large proportion of these emerging diseases, and our current understanding of their dynamics may be affected by changing global conditions including the discovery of novel pathogens. With multiple *Borrelia* spp. pathogens circulating in a common geographical area, the potential for antigenic cross-reactivities in immunoassays can complicate the diagnosis of tick-borne diseases like Lyme disease and *B. miyamotoi* disease (Krause et al. 2018). Thus, more detailed studies to describe the distribution and epidemiology of these pathogens are imperative to improve human health diagnoses.

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**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3171/full