AEM Accepted Manuscript Posted Online 7 August 2020
Appl. Environ. Microbiol. doi:10.1128/AEM.01118-20
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# Search for *Campylobacter* reveals high prevalence and pronounced genetic diversity of *Arcobacter butzleri* in floodwater samples associated with Hurricane Florence, North Carolina, USA

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Running title: *Arcobacter butzleri* in Hurricane Florence floodwaters

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**ABSTRACT (250 words)**

In September 2018, Hurricane Florence caused extreme flooding in eastern North Carolina, USA, a region highly dense in concentrated animal production, especially swine and poultry. In this study, floodwater samples (n=96) were collected as promptly post-hurricane as possible and for up to approx. 30 days, and selectively enriched for *Campylobacter* using Bolton broth enrichment and isolation on mCCDA microaerobically at 42ºC. Only one sample yielded *Campylobacter*, which was found to be *Campylobacter jejuni* with the novel genotype ST-2866. However, the methods employed to isolate *Campylobacter* readily yielded *Arcobacter* from 73.5% of the floodwater samples. The *Arcobacter* isolates failed to grow on Mueller-Hinton agar at 25, 30, 37 or 42ºC microaerobically or aerobically, but could be readily subcultured on mCCDA at 42ºC microaerobically. Multilocus sequence typing of 112 isolates indicated that all were *Arcobacter butzleri.* The majority (85.7%) of the isolates exhibited novel sequence types (STs), with 66 novel STs identified. Several STs, including certain novel ones, were detected in diverse waterbody types (channel, isolated ephemeral pools, floodplain) and from multiple watersheds, suggesting the potential for regionally-dominant strains. The genotypes were clearly partitioned into two major clades, one with high representation of human and ruminant isolates and another with an abundance of swine and poultry isolates. Surveillance of environmental waters and food animal production systems in this animal agriculture-dense region is needed to assess potential regional prevalence and temporal stability of the observed *A. butzleri* strains, as well as their potential association with specific types of food animal production.

**IMPORTANCE (150 words)**

Climate change and associated extreme weather events can have massive impacts on the prevalence of microbial pathogens in floodwaters. However, limited data are available on foodborne zoonotic pathogens such as *Campylobacter* or *Arcobacter* in hurricane-associated floodwaters in rural regions with intensive animal production. With high density of intensive animal production as well as



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pronounced vulnerability to hurricanes, Eastern North Carolina presents unique opportunities in this regard. Our findings revealed widespread incidence of the emerging zoonotic pathogen *Arcobacter butzleri* in floodwaters from Hurricane Florence. We encountered high and largely unexplored diversity while also noting the potential for regionally-abundant and persistent clones. We noted pronounced partitioning of the floodwater genotypes in two source-associated clades. The data will contribute to elucidating the poorly-understood ecology of this emerging pathogen, and highlight the importance of surveillance of floodwaters associated with hurricanes and other extreme weather events for *Arcobacter* and other zoonotic pathogens.

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## INTRODUCTION AND SPECIFIC AIMS

Hurricanes and other extreme weather events that can result in massive flooding of urban or agricultural areas have profound public health implications for contamination of surface waters (1–10). Chemical contaminants (e.g., heavy metals, antibiotics and other pharmaceuticals) can leak from overflowing, inundated or damaged sewage or animal waste containment structures into adjacent surface waters. Microbial agents, including pathogenic bacteria, viruses and parasites, can similarly become introduced into surface waters and persist on agricultural land and in urban areas. Such hazards are accentuated in rural areas with concentrated animal production, including concentrated animal feeding operations. However, relevant data remain sparse and incomplete, primarily due to impaired accessibility, safety considerations and accompanying delays in accessing and sampling impacted sites. There is a notable lack of reports that assess hurricane impacts on biological and chemical contaminants in floodwaters, and in the context of geospatial features. Observational data gaps related to microbial water quality in floodwaters has prevented investigation of questions related to the importance of dilution relative to increased exposure. Although flooding increases potential exposure of surface waters to microbes, the large volumes of water associated with flooding may also dilute microbial agents, in turn counteracting the effects of increased contaminant loading.

On September 12, 2018, Florence, a large, slow-moving hurricane, made landfall on the North Carolina coast, resulting in record-breaking flooding for several locations. In the seven days that followed, certain North Carolina communities received over 30 inches of rain, surpassing any of the previously-recorded amounts of rainfall from a single storm in the region and resulting in unprecedented flood magnitudes for many inland rivers (11). Such heavy rainfall and flooding can massively impact water quality and safety in flooded areas, especially via runoff from agricultural operations. Eastern North Carolina is highly dense in facilities that produce food animals, including swine and poultry, especially turkeys (12, 13). Swine production units with multiple houses and large numbers of animals

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in each house are highly prevalent in the region, with turkey and swine production frequently interspersed (14). Animal production is a leading source of employment for many of the region's residents. However, this region is also prone to a high frequency of severe weather events, including major hurricanes (15).

Hurricane Florence was preceded two years earlier by another major hurricane (Matthew, September 28 - October 10, 2016) with long-lasting adverse impacts on the socioeconomic landscape of North Carolina. Several of the Hurricane Florence-impacted areas had been previously flooded by Matthew. A research team in North Carolina had been assembled to investigate the environmental and public health impacts of Hurricane Matthew (16). Therefore, this team was already in place and readily poised to collect and analyze Hurricane Florence-associated floodwater samples as soon as it became logistically possible and safe to reach impacted areas. The original objective of the current study was to assess the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in the floodwaters, and allow comparisons with genotypic data collected over several years of investigation of these zoonotic pathogens in food animals and wildlife in this region (14, 17–25). However, in the course of the study we detected numerous samples positive for *Arcobacter*, and therefore we undertook the additional objective of characterizing the prevalence and genotypic diversity of *Arcobacter* from the hurricane-associated floodwaters.

## MATERIALS AND METHODS

**Water sample collection.** A total of 96 floodwater samples were collected at sites in the Neuse (n=39), Cape Fear (n=20), Lumbee (Lumber) (n=24), and Waccamaw (a sub-basin of the Lumbee) (n=13) watersheds in Eastern North Carolina (Table S1 and Fig. 1). The Neuse (Fig. 1A), Cape Fear (Fig. 1B) and Lumbee (Fig. 1C) are all distinct river basins, without surface connectivity between them. Water was collected in autoclaved one-liter Nalgene bottles triple-rinsed with the target sample water prior to collection. Flood sample collection sites were classified into four categories: 1, Channel i.e., flowing

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107 water in stream channels; 2, Floodplain i.e., slow-moving or stagnant floodplain water; 3, Isolated
108 ephemeral e.g., pools of floodwater likely to dry within a few days in the absence of additional rainfall;
109 and 4, Other, such as isolated permanent water bodies (i.e. ponds, lakes). Sampling was performed in
110 two distinct time periods, designated phase 1 and phase 2, yielding 50 and 46 samples, respectively.
111 Phase 1 sampling started within 7 days of Hurricane Florence's landfall and occurred between 9/18/2018
112 and 9/28/2018, while phase 2 sampling occurred on 10/18/2018 and 10/19/2018. Coordinates of each
113 sample location were recorded and logged using a handheld GPS unit and Google Earth. Efforts were
114 made to sample the same sites in both phases. In cases where the exact sample site from phase 1 was no
115 longer available (i.e., area was no longer flooded), samples were collected from a nearby similar location.
116 Additional samples were taken from the Lumbee watershed on 11/13/18 (n=2) and the Rocky Branch
117 Creek, in Raleigh, North Carolina, on 10/15/18 (n=4). Upon collection, the samples were immediately
118 stored in coolers on ice, transported to the laboratory, and stored at 4°C until processing, typically within
119 24-72 h.
120
121 **Isolation of *Campylobacter* and *Arcobacter*.** The majority of the samples (n=64) were processed via
122 parallel enrichments of water (1.3 ml), as well as 0.45 µm filters (Thermo Fisher Scientific, Inc.,
123 Waltham, MA) prepared via vacuum filtration of 50 ml water, while 34 samples were processed only via
124 enrichments of the water suspension. The filters were subsequently cut with flame-sterilized scissors into
125 three equal-size fragments, one of which was used for enrichment of *Campylobacter* (the remaining
126 fragments were utilized to enrich for *Salmonella enterica* and *Listeria* spp., which will be described in a
127 separate presentation). The water samples and filter fragments were enriched for *Campylobacter* in 10
128 ml Bolton Broth (Oxoid Ltd., Hampshire, UK) and incubated under microaerobic conditions at 37°C for
129 24 h using GasPak EZ Campy sachets (Becton, Dickinson and Co., Sparks, Maryland, USA). Following
130 enrichment, appropriate dilutions were prepared, 100 µl was spread-plated on modified charcoal-

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131 cefoperazone-deoxycholate agar (mCCDA; Oxoid), and the mCCDA plates were incubated
132 microaerobically at 42°C for 48 h, as described (17). An average of five putative *Campylobacter* colonies
133 per positive sample were transferred from mCCDA to Mueller Hinton Agar (MHA; Becton, Dickinson
134 and Co.) for purification following incubation microaerobically at 42°C for 48 h, as described (17). Many
135 cultures (found upon further analysis to be *Arcobacter*) grew poorly or not at all on MHA, and an average
136 of four *Campylobacter*-like colonies from such cultures were chosen and purified via subculture on fresh
137 mCCDA plates. Purified isolates were preserved at -80°C and *Campylobacter* species designations were
138 determined via multiplex PCR with *hip* and *ceu* primers to detect *C. jejuni* and *C. coli*, respectively, as
139 described (17). A subset of isolates that did not yield a *hip* or *ceu* amplicon via multiplex PCR were
140 analyzed via 16S rRNA sequencing (Genewiz, South Plainfield, NJ, USA) using the amplicon obtained
141 from the universal 16S primers 8f (5'-AGA GTT TGA TCC TGG CTC AG- 3′) (26) and 1492R (5'-
142 GGT TAC CTT GTT ACG ACT T- 3') (27).
143
144 **Multilocus sequence typing and minimum spanning trees.** *Campylobacter* or *Arcobacter* isolates
145 were chosen so as to represent each positive sample and enrichment type (suspension or filter) and
146 characterized via multilocus sequence typing (MLST) as described (28, 29). Novel *C. jejuni* and *A.*
147 *butzleri* alleles and sequence types were deposited into the corresponding PubMLST databases
148 (https://pubmlst.org/campylobacter/; https://pubmlst.org/arcobacter/). Concatenated sequences
149 representing all *A. butzleri* profiles within the PubMLST database, including those identified in this
150 study, were downloaded from PubMLST on November 14 2019 and again on July 13 2020. These
151 sequences were imported into BioNumerics (version 7.6.3; Applied Maths, Austin, TX) and aligned
152 using the Fast algorithm. Within BioNumerics, a Neighbor-joining dendrogram was constructed from
153 the aligned profile sequences; minimum spanning trees (MSTs) were constructed based on the sequence
154 distances between the concatenated profile sequences and using the default priority rules and

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'Permutation resampling' resampling strategy and 'Highscore summary' methods. MST nodes were color-coded within BioNumerics according to sampling date, source or location of isolation.

**Sequence Data**. All MLST sequence data have been deposited into PubMLST (https://pubmlst.org/campylobacter/; https://pubmlst.org/arcobacter/) as described above.

## RESULTS

***Campylobacter* was rarely detected in the floodwater samples, which instead frequently yielded *Arcobacter*.** Of the 98 water samples from the hurricane-impacted watersheds (96 from the floodwaters in phases 1 and 2 and two from the Lumbee basin three weeks later), only one (1.0%), a sample of channel water from the Waccamaw watershed in phase 2, was positive for *Campylobacter*. Several putative *Campylobacter* colonies from this sample were purified on MHA and all were found to be *Campylobacter jejuni*. MLST analysis of two of these isolates revealed a novel sequence type, ST-2866 (Table S1).

Interestingly, the enrichment procedures employed for *Campylobacter* yielded *Campylobacter*-like organisms from a large portion (72/98, 73.5%) of the samples. On mCCDA these *Campylobacter*-like cultures had a colony appearance suggestive of *Campylobacter*, and helical, motile cells were noted with phase contrast microscopy. However, unlike *Campylobacter* spp., these organisms grew poorly or not at all upon subculture on MHA or on tryptic soy agar with 5% sheep blood (Remel Microbiology Products, Lenexa, KS) and incubation at either 42 or 37ºC microaerobically, but could be readily subcultured on mCCDA at 42ºC microaerobically. Sequencing of PCR products obtained from a subset of isolates using 16S rRNA gene primers indicated 99% identity with *Arcobacter butzleri*. The genus *Arcobacter* has been proposed to be reorganized into to five novel genera, one of which, *Aliarcobacter* gen. nov., would include the species currently designated as *Arcobacter butzleri* (30). However, as



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179 discussed before (31), we consider the designation *Arcobacter* (for "aerotolerant campylobacters") valid,
180 pending a thorough phylogenomic assessment of Epsilonproteobacteria that would include
181 *Campylobacter*, *Helicobacter* and other genera, and therefore have chosen to maintain this taxonomic
182 designation in this work.

183 Putative *Arcobacter* was recovered frequently from enrichments of either water suspensions or
184 filters (63.1 and 79.7%, respectively). Of the 65 samples for which both water suspensions and filters
185 were enriched, 64.1% were *Arcobacter*-positive for both water and filters, while 3.1 and 16.9% were
186 positive only with the water suspension or the filter, respectively. Prevalence of *Arcobacter* was similar
187 in samples from phase 1 and phase 2 (72.0 and 73.9%, respectively) (Fig. 2). In each phase, the
188 *Arcobacter*-positive samples were distributed throughout the sampling region without any noticeable
189 spatial clustering within each sampled watershed, and were recovered with similar frequency from
190 samples of the two most prevalent waterbody types, i.e., channel (42/54, 77.8%) and floodplain (19/25,
191 76.0%) (Fig. 1). Total prevalence of *Arcobacter* across the two sampling phases was similar in the Neuse
192 and Lumbee watersheds (34/39, 87.2% and 21/24, 87.5%) followed by the Cape Fear (12/20, 60.0%) and
193 Waccamaw sub-basin (3/13, 23.1%).

194 ***Arcobacter butzleri* from floodwater samples exhibited high genotypic diversity, with several**
195 **genotypes isolated from multiple floodwater types, watersheds and sampling timepoints.** MLST
196 analysis of 112 putative *Arcobacter* isolates confirmed that all were *A. butzleri* and identified 74 STs, of
197 which 66 were novel (Table S1). The novel STs accounted for the majority (96/112, 85.7%) of the isolates
198 that were genotyped. Most of these novel STs were encountered just once among the floodwater isolates,
199 but several were detected in isolates from multiple samples (Fig. 3, Table S1). Even though different
200 colonies from the same enrichment typically had the same ST, different STs were frequently identified
201 in suspension vs. filter enrichments of the same sample (Table S1). Among the previously-identified

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202 STs we identified some that were shared with isolates of swine (ST-314), environmental water (ST-474),
203 ruminant (ST-138 and ST-750), poultry (ST-110 and ST-186), and human origin (ST-138) (Fig. 4).
204 Several (n=14) STs, of which all but two were novel, were identified in isolates from different
205 samples (Table 1). On several occasions the same ST was identified in different water types, timepoints,
206 and watersheds. Only two of these STs (ST-757 and 760, both novel) were encountered in just one
207 watershed (Neuse), each at different times during the sampling period (Table 1). Of the remainder, most
208 were from the Lumbee and at least one additional watershed, with two (ST-821 and 834) recovered
209 exclusively from watersheds other than the Lumbee. Certain STs detected in 3 or more samples were
210 noteworthy in their distribution. For instance, the novel ST-730 was identified in ephemeral water bodies
211 and channel water on two different dates spanning one month, and in both the Cape Fear and Lumbee
212 watersheds. ST-746, also novel, was isolated from channel samples in all four watersheds across the two
213 sampling phases, spanning an entire month. STs 734 and 750 were found in ephemeral, floodplain and
214 channel samples from the Lumbee as well as the Neuse watersheds on three different dates, again
215 spanning a whole month (Table 1 and Fig. 3).
216 **Floodwater *A. butzleri* genotypes partitioned in two major clades with different source-associated**
217 **compositions.** All but two of the 112 *A. butzleri* STs from the floodwater isolates were partitioned in
218 two major clades, designated cluster A and B (Fig. 4). The exceptions were ST-138 and ST-740, which
219 were localized in a different clade, designated cluster C (Fig. 4). The majority of floodwater isolates
220 grouped in cluster B (88/112, 78.6%), followed by cluster A (22/112, 19.6%). Source distribution
221 analysis including the other STs available in the *A. butzleri* PubMLST database revealed that cluster A
222 was highly populated by isolates of human and ruminant origin, with notable under-representation of
223 poultry or swine-derived isolates (Fig. 4 and Table S2). The opposite was found in cluster B, where
224 floodwater isolates were closely related to others of poultry and swine origin (Fig. 4 and Table S2). Only
225 one of the 112 genotyped floodwater isolates, from cluster B, shared its ST (ST-474) with an isolate

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previously obtained from environmental water (Fig. 4 and Table S2). Isolates from environmental water (outside of the floodwater isolates in the current study) were relatively uncommon in either cluster, and were mostly found in cluster C (Fig. 4 and Table S2) that included two of the floodwater STs (ST-138 and ST-740) from the current study. Isolates of human and food animal origin were also well-represented in cluster C (Fig. 4 and Table S2). None of the floodwater isolates from the current study mapped within another major cluster (designated D in Fig. 4) which included multiple STs from foods and food animals (Fig. 4).

Even though certain repeatedly-encountered STs were isolated from multiple watersheds and waterbody types (Table 1), cluster composition suggested potential dependence on watershed. Isolates from the Cape Fear watershed composed similar portions of both cluster A and B (13.6 and 15.9%, respectively), similarly to those from the Lumbee watershed (27.3 and 30.7%, respectively). However, more disproportionate contributions to cluster A and B were noted for isolates from the Neuse watershed (59.1% and 46.6%, respectively). Furthermore, Waccamaw isolates, albeit relatively few (n=6, with three different novel STs), were all found in cluster B, making up approx. 6.8% of the floodwater isolates in that cluster. Both STs in cluster C were from the Neuse watershed.

Geographically, cluster A consisted mostly of isolates from the United States (many from the current study), Thailand (primarily human) and the United Kingdom (primarily ruminant) (Fig. 4 and Fig. 5; Table S2). In contrast, cluster B had a significant representation of isolates from the US (primarily from the current study) and from Spain (primarily poultry, seafood, and other foods) (Fig. 4 and Fig. 5; Table S2). US isolates outside of the current study tended to be of human origin (Fig. 4 and Fig. 5; Table S2).

## DISCUSSION

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249 Even though surface water is considered a major source of pathogens that can contaminate the food
250 supply, little is known about the prevalence or genotypes of *Campylobacter* and *Arcobacter* in
251 floodwaters associated with extreme weather events such as hurricanes. Moreover, the lack of data on
252 pathogen presence in floodwaters limits our understanding of whether floodwaters have high microbial
253 contaminant concentrations due to increased surface water contact with contaminant sources, or if the
254 large volumes of water associated with floodwaters ultimately dilute microbial agents and result in low
255 contaminant concentrations. Consequently, estimates of public health risks associated with surface
256 waters in flood and post-flood conditions remain imprecise. The current study is, to our knowledge, the
257 first such report on the prevalence and genotypes of *Campylobacter* and *Arcobacter* in hurricane-
258 associated floodwaters. Our findings suggested that *Campylobacter* were uncommon (only one sample,
259 1.0%), while the methods employed for *Campylobacter* yielded *Arcobacter* from the majority (73.5%)
260 of the samples.

261 As indicated above, reports on *Arcobacter* prevalence in hurricane-associated floodwaters have
262 been lacking. However, *Arcobacter* contamination of groundwater subsequent to extreme precipitation
263 events was previously implicated in a massive waterborne outbreak in the Lake Erie region (32). Of the
264 16 groundwater wells surveyed in that study, seven were found positive for *Arcobacter*. *Campylobacter*
265 was not detected, but *Arcobacter* spp. were recovered on the selective media employed for
266 *Campylobacter* (32), as was also the case in our study. Unfortunately, the species or genotypes of
267 *Arcobacter* involved in that outbreak and groundwater contamination were not determined (32).

268 In our study, all analyzed *Arcobacter* isolates were found to be *A. butzleri*, an emerging water-
269 borne pathogen which has also been repeatedly isolated from diverse types of food (33–41). The media
270 and relatively high temperature employed here (42ºC) may well have prevented the recovery of other
271 *Arcobacter* species that may have been present in the floodwaters. In several studies, however, *A.*
272 *butzleri* was one of the most commonly-isolated *Arcobacter* species from water sources (39, 42–45).

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273 The prevalence of *Arcobacter*-positive samples in our study was high (73.5%), even though the
274 selective media and conditions were those intended for *Campylobacter*. A previous study utilizing
275 *Arcobacter*-specific selective media reported a similar prevalence (75%) from a river catchment in
276 Spain in autumn and winter, with higher prevalence in the spring and summer (45). Analysis of river,
277 sewage water and spring water in Turkey, utilizing *Arcobacter*-specific media, revealed *Arcobacter*
278 prevalence of 52, 36.4 and 12.5%, respectively (44). Our study might have revealed an even higher
279 prevalence had *Arcobacter*-specific media been employed. However, a rigorous analysis of a panel of
280 *A. butzleri* strains for growth on mCCDA vs. cefoperazone amphotericin teicoplanin (CAT) agar
281 designed for *Arcobacter* indicated that all strains could grow on mCCDA, even though some grew
282 better on CAT (46). In future studies, use of both mCCDA and CAT or other *Arcobacter*-specific
283 media will be valuable to maximize the chances of recovery of both *Campylobacter* and *Arcobacter*
284 spp. from floodwaters.

285 The identification of 74 STs among the 112 *A. butzleri* isolates that were genotyped from the
286 floodwaters suggests a highly-diverse population. The majority (66/74, 89.2%) of the STs from the
287 hurricane-impacted watershed samples were novel. This may reflect the fact that the *A. butzleri*
288 PubMLST database is still under-populated. In comparison to *Campylobacter* species such as *C. jejuni*
289 and *C. coli*, *A. butzleri* and other *Arcobacter* spp. remain much less commonly investigated and
290 genotyped. However, the findings may also reflect regional diversity in the population that we analyzed.
291 The *A. butzleri* PubMLST database lacked isolates from the same region as the floodwater isolates
292 investigated here, i.e., eastern and southeastern North Carolina.

293 The fact that several dominant STs (e.g., STs 460, 730, 734, 746 and 750; Table 1) were
294 encountered in isolates from multiple sample types, watersheds and timepoints that on certain occasions
295 spanned the entire month of sampling may reflect regionally-prevalent strains of *A. butzleri*. The
296 repeated detection of the novel ST-757 and 760 in only one watershed (Neuse) may reflect localized

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297 prevalence of the corresponding strains in that watershed, a speculation that will need to be addressed by
298 further sampling in the Neuse and other watersheds. On the other hand, the repeated detection of the
299 several STs in multiple watersheds that lacked surface connectivity (Table 1) suggests widespread
300 distribution of the corresponding strains in the region. In this context, it is of interest that *A. butzleri*
301 isolated during the same time period from Rocky Branch Creek, an urban creek in Raleigh, NC, had STs
302 which differed from those recovered from the floodwater samples but were still localized in clusters A
303 and B (2 STs each) (Fig. 4). Continued analysis of *A. butzleri* from environmental waters will be critical
304 to elucidate the geographic and temporal distribution of the strains encountered in the floodwater
305 samples, in order to better understand transmission dynamics and inform management and mitigation
306 strategies.

307 Previously identified STs in the floodwater isolates from this study were shared with isolates of
308 swine, poultry or ruminants, from other countries. We currently lack information on the prevalence or
309 genotypes of *A. butzleri* in agricultural animals in the Hurricane Florence-impacted region. Such
310 information is needed to determine whether apparently dominant and persistent *A. butzleri* STs identified
311 in the floodwaters, e.g., STs 746 and 750, may also be prevalent in animals produced in this food animal-
312 dense region or in surface waters during non-flooded conditions.

313 Previous studies of turkey and swine farms in eastern North Carolina, as well as wildlife and
314 cattle in the same region, using the same culture conditions as employed here, revealed high prevalence
315 of *Campylobacter*; *Arcobacter* was not isolated from those samples, which yielded exclusively *C. jejuni*
316 or *C. coli* (14, 17–19, 21, 24, 25). In the floodwater samples analyzed in the current study *Campylobacter*
317 prevalence was low (1.0%), in contrast to the overall high prevalence (73.5%) of *Arcobacter*. Even
318 though this may be due to true scarcity of *Campylobacter*, especially considering the relatively low
319 volume of water that was analyzed, it may also reflect preferential recovery of *Arcobacter* from water
320 samples that may be contaminated with both *Arcobacter* and *Campylobacter*, or possibly higher relative

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fitness of *Arcobacter* in these samples. Major gaps currently exist in our understanding of the relative fitness of *Arcobacter* and *Campylobacter* in environmental water and feces from agricultural animals.

In conclusion, our analysis of Hurricane Florence-associated floodwater samples revealed that *Campylobacter* was uncommon, with *C. jejuni* detected only once, while *Arcobacter*, specifically the emerging waterborne pathogen *A. butzleri*, was frequently recovered employing media and conditions intended for *Campylobacter*. Genotyping via MLST revealed high genotypic diversity among the *A. butzleri* isolates and a multitude of novel STs. Several STs, including novel ones, were detected in multiple watersheds, diverse types of water (channel, isolated ephemeral pools, floodplain) and repeatedly over the project survey period, suggesting the potential for dominant, persistent clones. Genotyping clearly partitioned the floodwater-associated *A. butzleri* isolates into two major clades, one of which had high representation of human and ruminant isolates, while the other was highly populated by swine and poultry isolates. The phylogenetic relationships among these strains, and their relatedness among themselves and those from other sources will be enhanced by continued surveillance and higher-resolution genotyping, as may be allowed with whole genome sequencing which is currently being undertaken for the floodwater-derived *A. butzleri* strains. Such information will need to be complemented by currently-lacking data on prevalence or genotypes of *A. butzleri* in agricultural animals in the impacted region. The widespread prevalence of *A. butzleri* in floodwaters, despite the opportunity for dilution, may signal that surface waters pose risks to public health during flooded conditions. Further, given that samples were collected shortly after hurricane landfall and throughout the course of several weeks thereafter, results suggest that public health risks associated with surface waters may persist beyond the peak of flooding. Further work is needed to determine the prevalence and genotypes of *Arcobacter* and *Campylobacter* in the watersheds of this region during hurricane-associated flooding but also in the absence of severe weather events, so that an assessment of baseline levels of *Arcobacter* can be made. Data are also needed on baseline incidence of human *Arcobacter* infections in



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this region and potential increases during hurricane-associated flooding. Such data are lacking. *Arcobacter* infections are currently not reportable and likely are rarely diagnosed, especially in the affected region which is largely rural, low-income and generally underserved, with relative scarcity of clinics that would collect and analyze human diarrheal samples. All water samples were collected from water bodies in Tier 1 counties, a designation reflecting highest distress levels based on economic well-being metrics (47). There is a critical need for integration of surveillance of environmental waters for pathogens such as *Arcobacter* and *Campylobacter* with public health data on the incidence of waterborne gastrointestinal illness in the hurricane-impacted communities.

**ACKNOWLEDGEMENTS**

This work was partially supported by the International Life Sciences Institute (ILSI)North America, Food Microbiology Committee. ILSI North America is a public, nonprofit science foundation that provides a forum to advance understanding of scientific issues related to the nutritional quality and safety of the food supply. ILSI North America receives support primarily from its industry membership. ILSI North America had no role in the design, analysis, interpretation, or presentation of the data and results. We are also grateful to the College of Agriculture and Life Sciences and the Department of Food, Nutrition and Bioprocessing Sciences at North Carolina State University for partial support of this study. Field sampling efforts were funded by the National Science Foundation (Award Number CBET-1901588) and North Carolina State University's Hurricane Florence Recovery Effort Travel Fund. We thank Katherine L. Martin, Jocelyn R. Painter, Justine Neville, Rhyan Stone, Andrea Stewart, Jeff Currie and Andrew Sanders for assistance with sample collection. All members of our laboratory are thanked for their support and assistance.



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## FIGURE LEGENDS

**Fig. 1**

**Water types and sampling sites.**

**A.** Sample results for *Campylobacter* and *Arcobacter* over the two sampling phases are indicated in red (positive) and black (negative) and waterbody types are shown by the indicated symbols. Base map tile was from Stamen (Terrain style), with open-source data from OpenStreetMap and OpenStreetMap Foundation. Maps were created in R using the ggmap package. **B.** Distribution of the sampling sites by watershed. (A), Neuse River Basin; (B), Cape Fear River Basin; (C), Lumbee (Lumber) River Basin; and (D), Waccamaw Basin, a sub-basin of the Lumbee Basin. Samples positive and negative for *Arcobacter* are shown in red and black, respectively and waterbody types are shown by the indicated symbols. The sole *Campylobacter*-positive sample site is also indicated on the map. The blue lines correspond to major hydrographic features, and the gray shaded areas correspond to the river basins. Scale bars (in km) are included for A-D, and the location of the four watersheds in the reference map of the state of North Carolina, USA, is shown at the bottom right of the Figure. Map was created with R using open-source geospatial hydrography data accessed through the North Carolina, USA, Department of Environmental Quality (http://data-ncdenr.opendata.arcgis.com/datasets/major-river-basins). The Lumbee river designation is in accordance with an ordinance passed by the Lumbee Tribal Council calling on all parties to observe the river's ancestral name. County, state, and federal government utilize the designation "Lumber river", created by state legislation in the 19th century (48, 49).

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393 **Fig. 2**

394 **Prevalence of *Campylobacter* and *Arcobacter* in Hurricane Florence-impacted watershed samples**
395 **over the study period.** Sample collection and processing for *Campylobacter* and *Arcobacter* were
396 performed as described in Materials and Methods.

397

398 **Fig. 3**

399 **Genotype distribution of *A. butzleri* floodwater isolates in the different sampling periods.** The
400 MLST-based minimum spanning tree demonstrates the genotype distributions of *A. butzleri* isolated and
401 genotyped in this study (in colors other than gray) and all other *A. butzleri* in the *A. butzleri* PubMLST
402 database (gray). Each circle represents a different ST determined by MLST. The size of the circle
403 indicates the number of isolates with the corresponding ST, with the smallest circles corresponding to
404 one isolate. Closely-related STs are connected by thick black lines. Phase 1 (blue and gold): 18-20
405 September 2018; Phase 2 (red): 18-19 October 2018. Genotypes of isolates from Rocky Branch Creek
406 on 15 October 2018 are in turquoise. Genotypes of isolates from two additional samples of the Lumbee
407 watershed collected on 13 November 2018 are in pink. MLST analysis and minimum spanning tree
408 construction were done as described in Materials and Methods.

409

410 **Fig. 4**

411 **Relatedness of *A. butzleri* floodwater isolates to *A. butzleri* from different sources.** The MLST-
412 based minimum spanning tree demonstrates the genotype distributions of *A. butzleri* isolated and
413 genotyped in this study in the context of all other *A. butzleri* from diverse sources available in the *A.*
414 *butzleri* PubMLST database. Florence floodwater and Rocky Branch Creek isolates are in black and gray
415 respectively, and other sources are in various other colors, as indicated in inset. Major identified clades
416 A, B, C and D are indicated, with A and B, harboring all but two of this study's genotypes, exhibited in

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higher resolution on the right-hand side of the Figure. Numerical ST designations of the floodwater isolates are indicated inside the circles in each cluster (A and B) inset on the right. The two STs (ST-138 and 740) outside of A or B are shown in cluster C (left-hand side of Figure). Each circle represents a different MLST-based ST. The size of the circle indicates the number of isolates with the corresponding ST, with the smallest circles corresponding to one isolate. Closely-related STs are connected by thick black lines. MLST analysis and minimum spanning tree construction was performed as described in Materials and Methods.

**Fig. 5**

**Relatedness *A. butzleri* floodwater isolates to *A. butzleri* from different countries.** The MLST-based minimum spanning tree demonstrates the genotype distributions of the floodwater isolates in the context of *A. butzleri* from different countries. *A. butzleri* isolated and genotyped in this study are in gray, while other isolates from the United States are in black. Diverse colors are used for other countries, as shown in inset. Included are all *A. butzleri* isolates in the *A. butzleri* PubMLST database. Each circle represents a different MLST-based ST. The size of the circle indicates the number of isolates with the corresponding ST, with the smallest circles corresponding to one isolate. Closely-related STs are connected by thick black lines. U.S. isolates previously outside of those in the current study are in black.

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580 **Table 1.** ***A. butzleri*** **STs identified in multiple samples**
581

| ST (No. samples, ST cluster) [1] | Date (No. samples) [2] | Waterbody type (No. samples) [3] | Watershed (No. samples)[4] |
|---|---|---|---|
| **721** (2, A) | 18 Sep (1), 18 Oct (1) | 1 (2) | L(1), N(1) |
| **736** (2, A) | 18 Sep (1), 18 Oct (1) | 1 (1), 2 (1) | L(1), N(1) |
| 460 (3, B) | 18 Sep (3) | 1 (2), 4 (1) | L (2), CF (1) |
| **726** (2, B) | 18 Sep (2) | 1 (1), 2 (1) | L (1), CF (1) |
| **729** (2, B) | 18 Sep (2) | 1 (1), 3 (1) | L (1), CF (1) |
| **730** (3, B) | 18 Sep (2), 18 Oct (1) | 1 (1), 3 (2) | L (1), CF (2) |
| **734** (3, B) | 18 Sep (2), 18 Oct (1) | 1 (1), 2 (1), 3 (1) | L (1), N (2) |
| **746** (6, B) | 18 Sep (2), 28 Sep (1), 18 Oct (2), 13 Nov (1) | 1 (5), LB (1) | L (3), CF (1), N (1), W (1) |
| 750 (5, B) | 18 Sep (2), 28 Sep (1), 18 Oct (2) | 1 (1), 2 (3), 3 (1) | L (1), N (4) |
| **757** (2, B) | 18 Sep (1), 18 Oct (1) | 1 (1), 3 (1) | N (2) |
| **760** (2, B) | 28 Sep (1), 18 Oct (1) | 1 (2) | N (2) |
| **821** (2, B) | 18 Oct (1), 19 Oct (1) | 2(1), 3 (1) | CF (1), W (1) |
| **824** (2, B) | 18 Oct (2) | 2 (1), NA (1) | CF (1), N (1) |
| **827** (2, B) | 18 Oct (1), 13 Nov (1) | 2 (1), LB (1) | L (2) |

582

583 [1] Novel STs are in bold font. Clusters are as in Table S1 and Fig. 3.
584 [2] Dates are all in the year 2018.
585 [3] Waterbody types, as in Table S1. 1, Channel; 2, Floodplain; 3, Isolated ephemeral; 4, Other (large
586 pond); NA, information not available; LB, Lumbee Basin, collected post-phase 2 on 11 Nov.
587 [4] Lumbee; N, Neuse; CF, Cape Fear; W, Waccamaw, as in Fig. 2. Detailed information on the
588 coordinates of the samples is present in Table S1.

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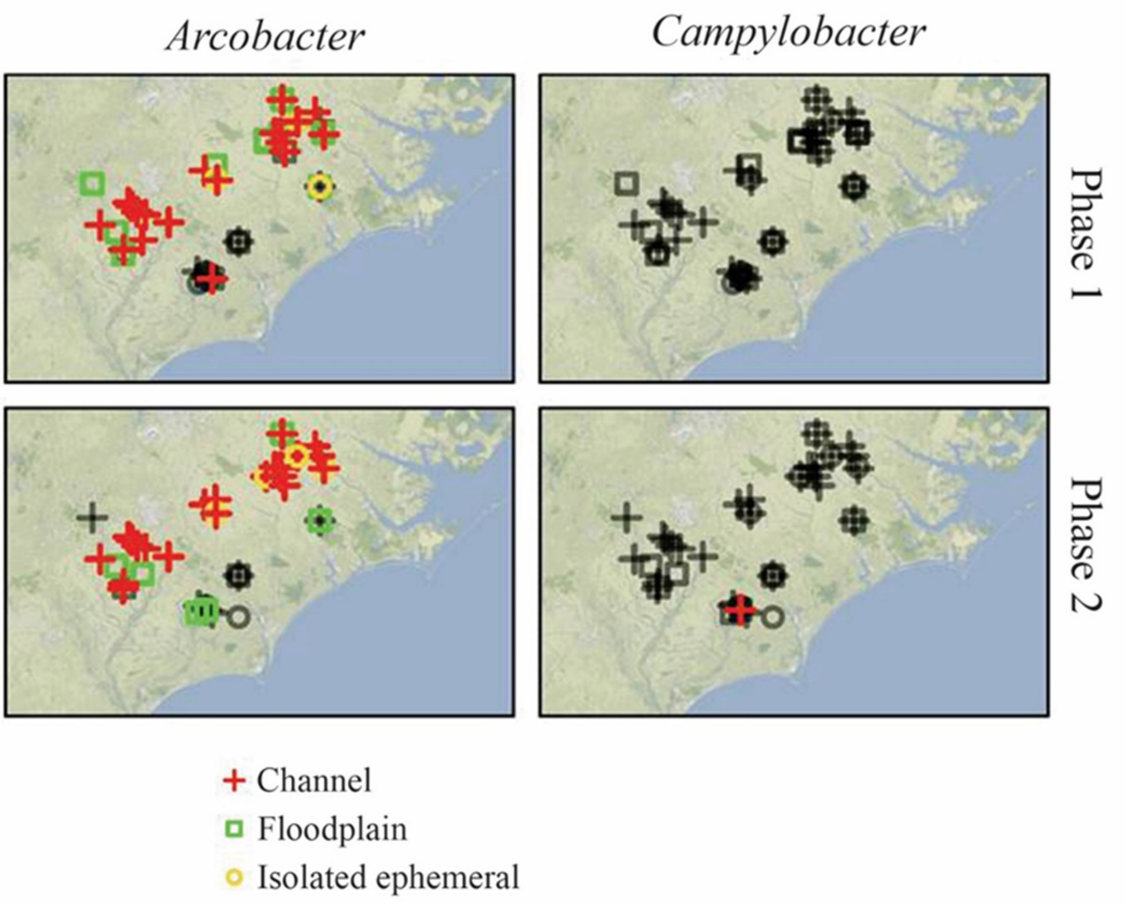
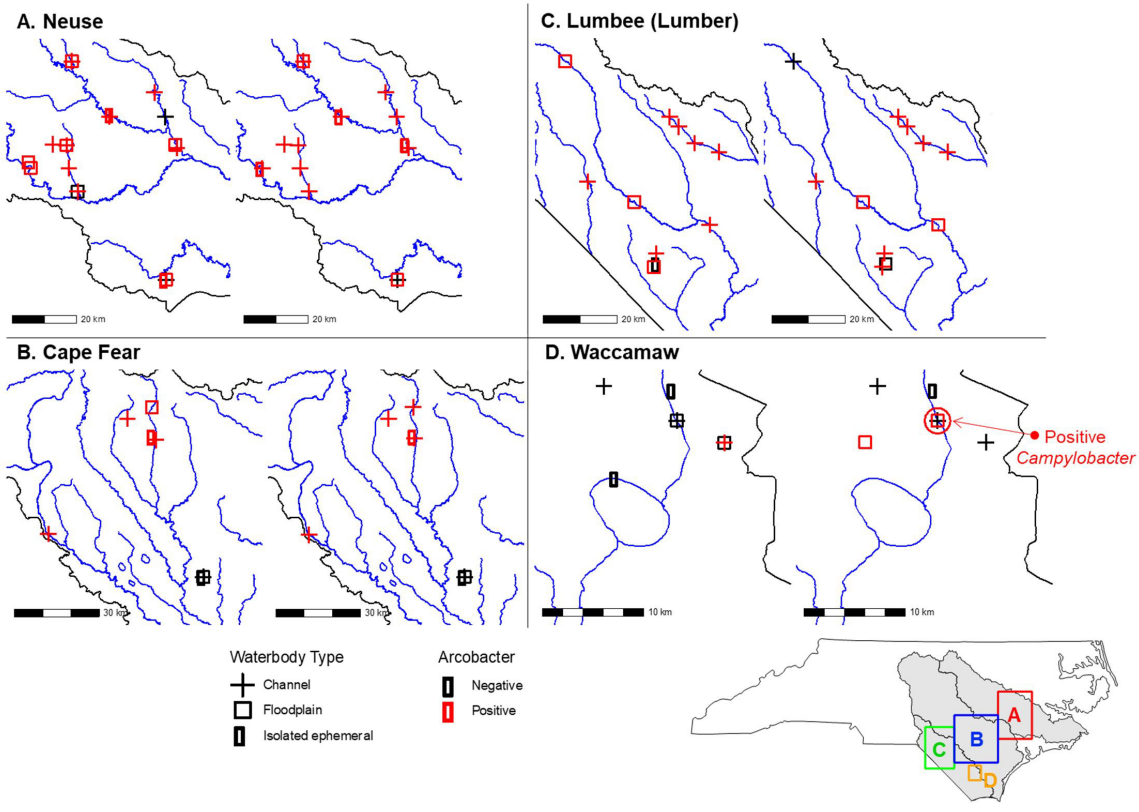
**A.**




**B.**

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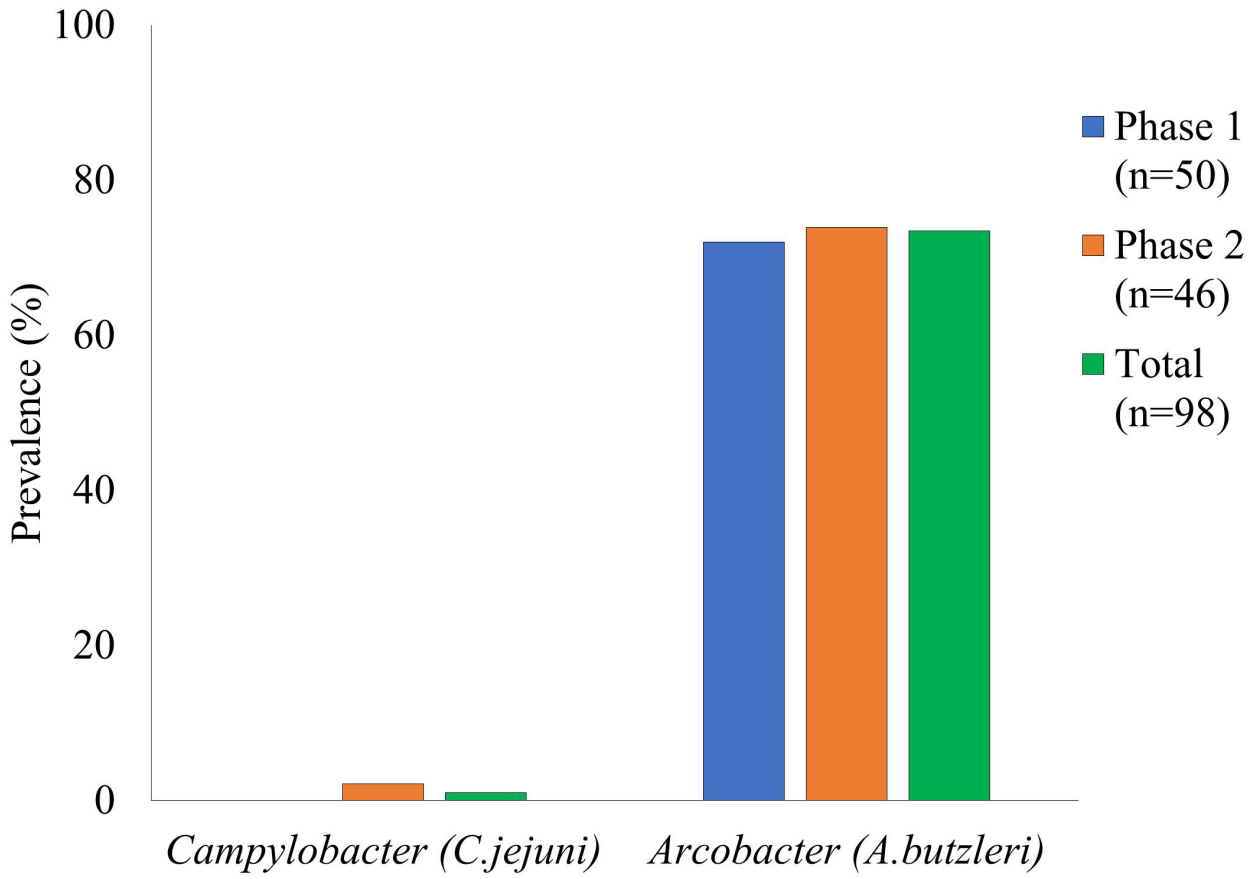


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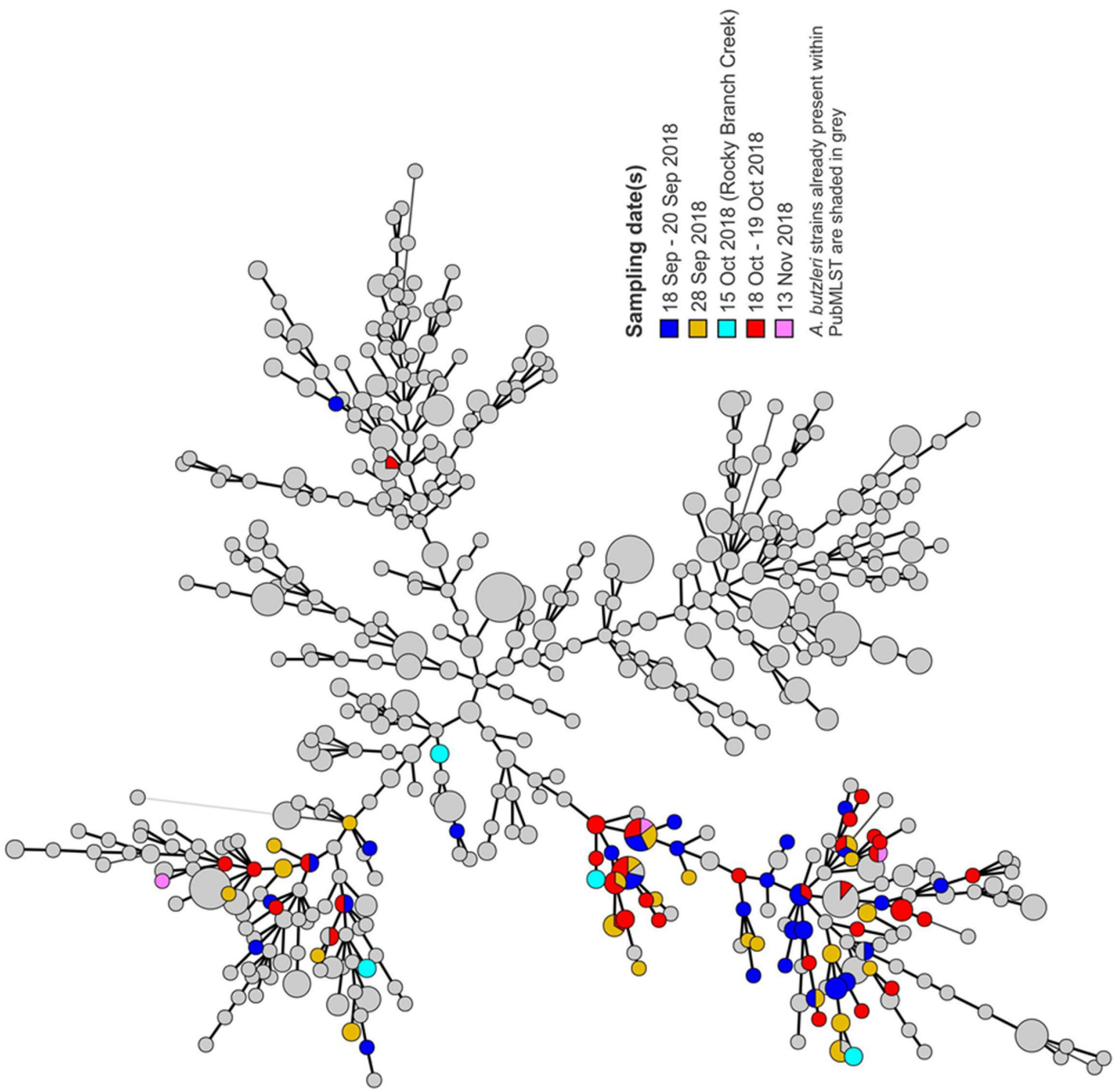


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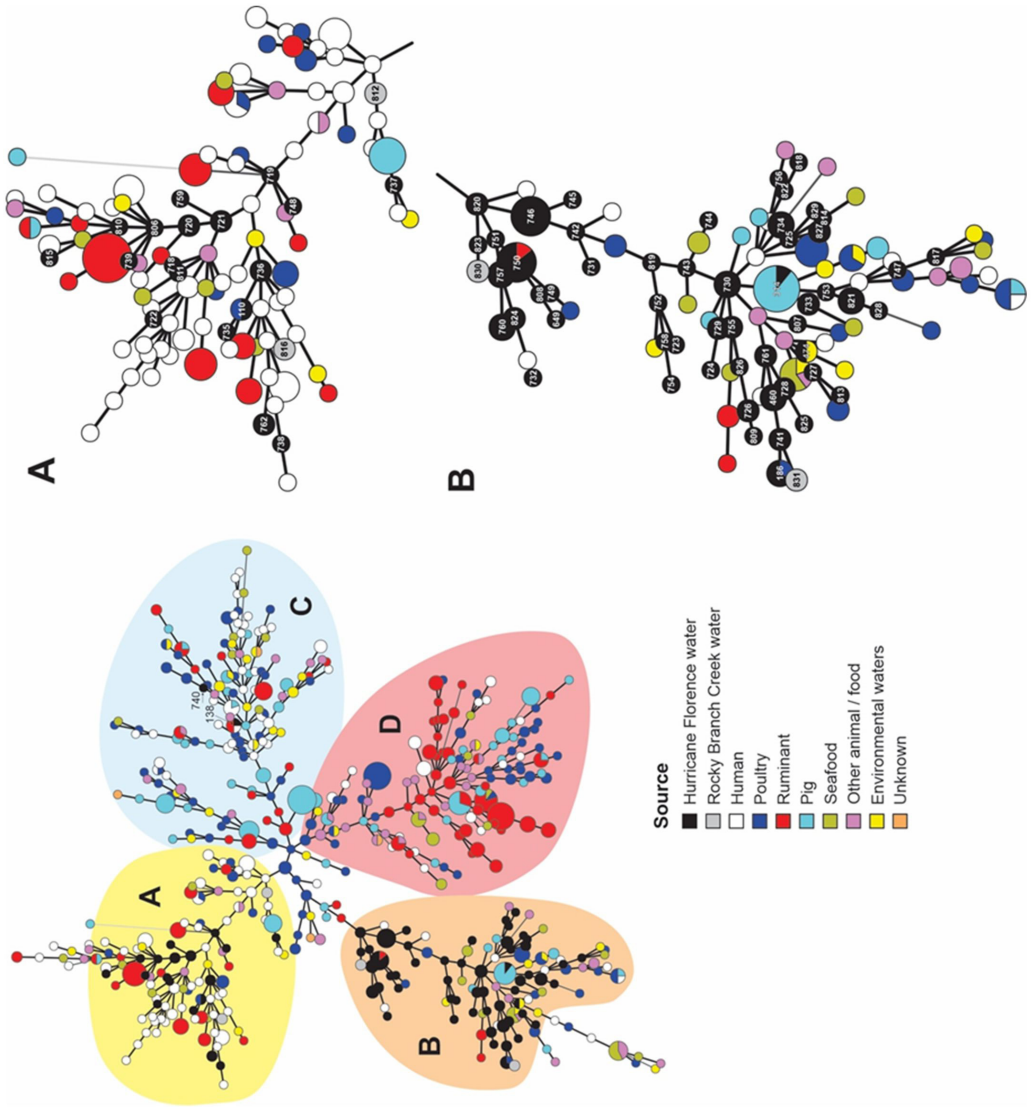


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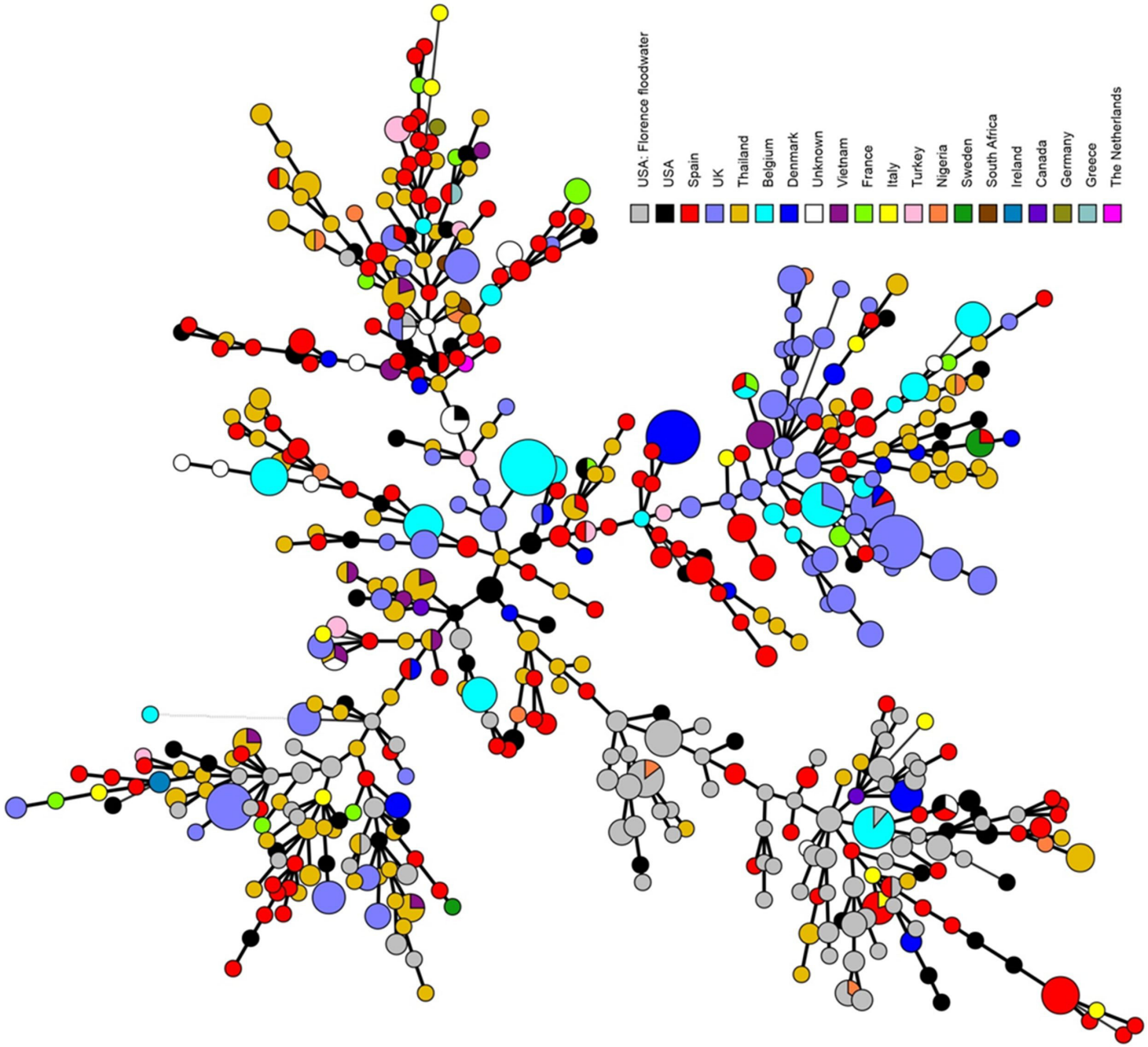


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