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REVIEW

The Hop Cyst Nematode, *Heterodera humuli*: History, Distribution, and Impact on Global Hop Production

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Abstract

Humulus lupulus (commonly known as hop) is an herbaceous plant that is used in brewing throughout the world. Hop cones are an essential ingredient in the production of beer, which makes hops of critical importance to global craft beverage industries. The hop cyst nematode, *Heterodera humuli*, is a plant-parasitic nematode with the potential to substantially limit yields of hop. *H. humuli* has been detected in many of the most significant regions for hop production worldwide, and infestations of *H. humuli* can consequently impact hop growth and limit cone production. Despite documented reports on the distribution of and damage caused by *H. humuli* since its description in 1934, there have been limited studies on the biology, pathogenicity, management, and consequences of infestations on hop production over time. Inconsistencies and gaps in the available information (e.g., the number of *H. humuli* generations per season, host status of alternate crops), exacerbate difficulties in understanding how *H. humuli* can be managed. Resolving the existing knowledge gaps identified within this review can lead to determining effective *H. humuli* management strategies for hop growers.

Keywords: distribution, *Heterodera humuli*, hop cyst nematode, hops, identification, management

History of Hop Production Globally

Beer is one of the oldest and most important beverages globally (De Simone et al. 2021). In 2020, the global beer market was valued at U.S. $623.2 billion (IMARC 2021). Different herbaceous plants have been used to bitter and flavor beer; among them, hop (*Humulus* spp.) are the most prominent plant used. There are three species commonly known as hop: *Humulus yunnanensis*, *Humulus scandens*, and *Humulus lupulus*. However, *Humulus lupulus* (hereafter referred to as hop) is the only species that has been considered an essential ingredient in brewing (Bamforth 2017; Kovalchuk et al. 2020; Moir 2000).

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Hop is a dicotyledonous, perennial, herbaceous plant that belongs to the Cannabaceae family. Its cones are an essential ingredient in beer production to add bitterness and flavor (Murakami et al. 2006; Šrédl et al. 2020). The use of hop in brewing dates back to the first evidence of hop as a cultivated plant, between the eighth and ninth century (Edwardson 1952). Hop production started in Central Europe, in Hallertau, Bavaria, Germany, and gradually spread to Belgium, England, Sweden, Bohemia (currently Czech Republic), Slovenia, and other temperate countries across Europe (Edwardson 1952; Kopp 2014; Neve 1991). More than 10 European countries currently produce hop commercially, of which Germany and the Czech Republic account for a total of 33 and 8% of worldwide hop production, respectively (Hop Growers of America 2022).

From Europe, hop cultivation spread to other continents such as Australia and America. The first attempt to introduce hop into Australia was in 1803, in New South Wales. There, private growers were able to cultivate a limited supply of hop. The first brewery was founded in 1804 but depended mainly on imported hop supplies. This resulted in the halt of hop production in the 1850s. Hop production was much more successful in Tasmania. The first breweries were established in Hobart, Tasmania in the early 1820s. In 1822, William Shoobridge was granted 8.1 ha of land to plant hop, but it was not until 1825 when he was able to produce the first marketable crop (Evans 1993). As of 2021, Australia had approximately

787.1 ha of active hop production (Hop Growers of America 2022) with the majority of production in Tasmania (John 2010).

In the United States, the first cultivated hops were introduced around 1629 by the Massachusetts Bay Company (Edwardson 1952). Until the late 1830s, New England states were the most important region of hop production in the United States. By 1859, New York accounted for 90% of hop production in the United States (Krakowski 2014; Oliver 2012; Parsons 1940). By 1870, Wisconsin and Michigan also emerged as important hop producers, but New York was still the leader of U.S. hop production (Barnett 2020). In 1880, the Pacific Coast (California, Oregon, and Washington) accounted for less than 10% of the national production (Parsons 1940). However, ongoing problems with pests and diseases such as aphids (*Phorodon humuli*) and mildews (*Golovinomyces orontii* and *Peronospora sparsa*) led to a decrease in hop production in New York, resulting in the Pacific Coast becoming the main producer of hops in 1893 (Parsons 1940). By 1920, there was no longer commercial hop production on the east coast (Edwardson 1952; Oliver 2012; Parsons 1940).

Currently, the United States is the world's largest producer of hop, with 25,195 ha; Pacific Northwest (PNW) growers account for 98% of this production (Hop Growers of America 2022). Washington accounts for 73% of the U.S. hop production, followed by Idaho and Oregon with 16 and 11%, respectively. More than 50% of the U.S. hop production relied on six hop varieties: Citra, Mosaic, Columbus/Tomahawk/Zeus (CTZ), Cascade, Simcoe, and Centennial (USDA-NASS 2021).

Like many other crops, hop production faces challenges due to pests and diseases. Downy mildew (*Pseudoperonospora humuli*), powdery mildew (*Podosphaera macularis*), and verticillium wilt (*Verticillium nonalfalfae* and *V. dahlia*) are major diseases impacting hop in the PNW. Plant-parasitic nematodes can also impact the production of hop. In particular, the hop cyst nematode, *Heterodera humuli,* is a pest frequently found in hop yards (Warner et al. 2015). This review summarizes the knowledge gained about *H. humuli* distribution, biology, identification, host status, and control since its initial discovery, and highlights the existing knowledge gaps within these sections.

Worldwide Distribution of *H. humuli*

H. humuli was first encountered in 1894 from hop yards within the south and west regions of England, although the nematode was originally misidentified as the sugar beet cyst nematode, *H. schachtii*. At the time, it was suspected that this nematode was the disease agent in hop Nettlehead, which piqued interest as part of a potential disease triangle (De Grisse and Gillard 1963; Stone and Rowe 1977). Nettlehead is now known to be a virus transmitted by aphids (Duffield 1925), and due to the lack of obvious symptoms of disease, *H. humuli* was largely forgotten for a number of years (Stone and Rowe 1977). Descriptions of this nematode by Voigt and Triggitt led to the official description of *H. humuli* 40 years later by Filipjev in 1934 (Sveshnikova 1956). It was not until a few decades later that *H. humuli* was first recorded in Germany (1951), followed by Israel (1956) (Franklin 1951). In 1957, Gillard and Van de Brande first reported *H. humuli* in Belgium, and by 1963, 100% of sampled Belgium fields contained hop cysts (De Grisse and Gillard 1963).

Other regions where *H. humuli* has been reported include Australia, Bulgaria, Canada, Croatia, Czech Republic, Greece, Holland, India, New Zealand, Poland, Russia, Sweden, Switzerland, Slovakia, Slovenia, Ukraine, and the United States (Danilova 1996; Hržič 1986; Katalan-Gateva and Konstantinova-Milkova 1975; Lišková and Renčo 2007; Renčo et al. 2011; Stone and Rowe 1977). During a soil survey in the regions of Waboomskraal, Herold, and Outeniqua of South Africa, *H. humuli* was first reported and recovered from 42% of surveyed hop yards (Malan et al. 1991). *H. humuli* collected from common nettle, *Urtica dioica*, was recently reported in Turkey (Akyazi et al. 2018). Interestingly, despite several hop surveys and its widespread distribution in Europe, *H. humuli* has not been recovered in Italy (Landi et al. 2019). Overall, the worldwide distribution of *H. humuli* spans five of the seven continents (Fig. 1).

Nematodes collected from preserved soil debris collected from an 840 AD well in England were identified as *H. humuli* cysts in the presence of *U. dioica* plant material and seeds. This was a prominent finding in understanding the origin of *H. humuli* distribution because hop was not introduced to England until the 16th century. The historic cysts were compared with *H. humuli* cysts from 1974 and confirmed to be the same species, adding evidence that suggests *U. dioica* could have been the original primary host of *H. humuli* (Webley 1974).

H. humuli Distribution Within the United States

As previously mentioned, the first documented appearance of *H. humuli* in the United States occurred in early spring of 1962 in Pierce County, Washington. Cysts were obtained from hop cuttings sent with infested soil from Canada, and following this discovery, Pierce County hop yards were surveyed and identified to contain the nematode (Cobb 1962). Cobb noted that due to the lack of characteristic symptoms of infested yards, *H. humuli* may have existed in PNW soils for an undetermined number of years.

Shortly after its initial discovery in Washington, *H. humuli* was also recovered from Oregon hop yards (Jensen et al. 1962). As of writing this review, Arun K. Sen was the only graduate student to receive their Ph.D. for a project relating to the biology of *H. humuli*, and his dissertation documents the majority of what is currently known about optimal conditions, host range, and pathology of the nematode (Sen 1968). *H. humuli* research in the United States remained largely dormant until its detection in Idaho in two multicrop field surveys (Hafez et al. 1992; Hafez et al. 2010). Thereafter, in 2012, an extension specialist reported a heavily impacted hop yard in the Old Mission Peninsula of Michigan that yielded an average of 241 cysts/100 cm^3 of soil sample (Warner et al. 2015). These four states are also the top four hop-producing states in the United States, but as mentioned previously, distribution likely extends further.

Since its initial discovery in Washington, little work has been conducted to document distribution of *H. humuli* in hop-growing regions of the United States. Recently, *H. humuli* was discovered to be widespread in the most significant hop production region in Washington, Yakima Valley, where nearly 75% of U.S. hop production occurs (Darling et al. 2021). This finding highlights the threat that *H. humuli* poses to U.S. hop production.

Morphology and Identification of *H. humuli*

The identification of plant-parasitic nematodes to the species level is an important step in establishing strategies to combat them. Morphological and morphometric features can be used to achieve identification (Abdollahi 2008; Dawabah et al. 2012). The shape of the cyst is a characteristic that allows separation between cyst nematode genera. *Heterodera* is typically characterized by lemon-shaped cysts with a prominent vulval cone (Fig. 2A). These features allow for differentiation from other cyst nematodes such as *Globodera*, which has rounded-cysts without vulval cone (Franco 1986; Skantar et al. 2011; Subbotin et al. 2010a, b), from *Dolichodera* and *Paradolichodera*, that do not have vulval cone, and from *Cactodera,* which has a small vulval cone (Moens et al. 2018).

Within the genus *Heterodera*, vulval cones can be classified based on the type of fenestration: circunfenestrate, ambifenestrate, and bifenestrate (Turner and Subbotin 2013). Based on morphological and molecular characteristics, species of *Heterodera* have been divided into nine groups, i.e., *Afenestrata*, *Avenae*, *Bifenestra*,

Cardiolata, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari*, and *Schachtii* (Handoo and Subbotin 2018). *H. humuli* belongs to the *Humuli* group, characterized by its bifenestrate vulval cone (Fig. 2B) and the presence of a weak vulval bridge (Subbotin et al. 1997). These characteristics help differentiate *Humuli* from other groups such as *Schachtii*, which has ambifenestrate vulval cones with a developed underbridge (Mulvey and Golden 1983), *Afenestrata*, where vulval cones are not fenestrated (Baldwin and Bell 1985), and *Sacchari*, which have vulval cones with a strong underbridge and the presence of finger like projection (Maafi et al. 2007).

Cysts of *H. humuli* are small (418 to 540 µm) (Maafi et al. 2004; Sen and Jensen 1969) and can be differentiated from *H. fici* by type of fenestration (bifenestrate versus ambifenestrate), which is an exception within the *Humuli* group (Golden et al. 1988; Sen and Jensen 1969). Cysts of *H. humuli* can contain up to 338 eggs (Fig. 2C) (Renčo et al. 2011; Von Mende and McNamara 1995a). Eggs of *H. humuli* are smaller than eggs of *H. litoralis*, with average lengths of 94 to 98 µm versus 130 µm and average widths of 38.5 µm versus 45 µm (De Grisse and Gillard 1963; Stone and Rowe 1977; Wouts and Sturhan 1996).

Second stage juveniles (J2) of *H. humuli* have a strong stylet and cephalic region (Fig. 2D and E) and differ from J2 of other species within the *Humuli* group, such as *H. ripae* with a larger tail (49 to 50 µm versus 40 to 47 µm) and hyaline region (26.3 to 29.3 µm versus 19 to 23 µm) (Fig. 2F) (Maafi et al. 2004; Wouts and Weischer 1977) and from *H. litoralis* with smaller average body length (364 to 425 µm versus 520 µm) and average stylet length (22.5 to 24 µm versus 29.5 µm) (De Grisse and Gillard 1963; Stone and Rowe 1977; Wouts and Sturhan 1996).

Humuli Group (molecular classification)

The identification of plant-parasitic nematodes to the species level based upon morphology is time-consuming and requires the skills of trained nematode taxonomists (Mundo-Ocampo et al. 2008). Currently, molecular diagnostics can be used as a complementary tool to the morphological characterization of nematodes to provide accurate identification in a shorter period of time and with a reduced skill set (Subbotin et al. 2000; Szalanski et al. 1997).

Polymerase chain reaction (PCR), PCR species-specific primers, PCR-restriction fragment length polymorphism (PCR-RFLP), real-time PCR (RT-PCR), and quantitative PCR (qPCR) are some examples of DNA-based molecular techniques that have been developed for plant-parasitic nematode identification, including cyst-forming nematodes (Mundo-Ocampo et al. 2008; Weayenberge et al. 2009; Ye 2012). Some of these techniques have been used to separate *H. humuli* from closely related species within the *Humuli* group. For example, the use of the enzyme *Alu*I in PCR-RFLP of the internal transcribed spacer (ITS) region of *H. humuli* yields a unique four-band pattern (451, 241, 175, and 171 bp) that distinguishes it from *H. fici* (756, 174, and 105 bp) and *H. ripae* (628, 243, and 178 bp) (Maafi et al. 2003; Madani et al. 2004).

Phylogenetic relationship is another molecular approach that has been used to identify cyst-forming nematodes. The D2-D3 expansion segments of the 28S gene (*28S*) and the *ITS1*-5.8*S*-*ITS2* both from ribosomal DNA, and the partial *cox1* gene (*cox1*) from the mitochondrial genome are commonly used to identify closely related species within the genus *Heterodera* (Bernard et al. 2010; Madani et al. 2004; Mundo-Ocampo et al. 2008; Sekimoto et al. 2017; Skantar et al. 2011; Subbotin et al. 2010a). The *cox1* and rDNA genes have multiple copies in nematode genomes, allowing amplifications to be obtained from a single nematode (Bogale et al. 2020). However, just two rDNA genes, the *ITS* (Fig. 3) and the *28S* have been used to perform phylogenetic inferences to separate *H. humuli* from closely related species within the *Humuli* group such as *H. fici*, *H. litoralis*, *H. ripae*, *H. turcomanica*, and *H. vallicola* (Fanelli et al. 2019; Madani et al. 2004; Subbotin et al. 2021).

FIGURE 1
Current known worldwide distribution of the hop cyst nematode, *Heterodera humuli*, based on first reports (1894 to 2022). Created using Biorender (https://biorender.com/).

Impact of *H. humuli* on Crop Physiology and Growth

H. humuli is a sedentary endoparasitic nematode and its life cycle relies on cues at different developmental stages of its host. In winter, eggs of *H. humuli* are protected within the dead female's hardened body (cyst). In spring, when hops begin to sprout, root exudates stimulate hatching of *H. humuli* eggs. *H. humuli* J2 penetrate and migrate into hop roots to find cells near the vascular tissue to establish a feeding site (syncytium), from where the nematode will obtain the nutrients to complete its life cycle (De Grisse and Gillard 1963; Hay and Pethybridge 2003; Von Mende and McNamara 1995a). It has been reported that *H. humuli* may have one or two generations (Fig. 4) in a year (Nuber 1958; Mikhailpukov 1980; Simon 1958; Von Mende and McNamara 1995a). However, there is no consensus on this. *H. humuli* is one of the few plant-parasitic nematodes that can be observed in the field; white females can be seen on the root surface because its body ruptures the root cortex once the mature female swells (Smiley et al. 2017).

Root-feeding by most plant-parasitic nematodes does not produce specific symptoms (Jones et al. 2013). However, nematode parasitism can reduce the roots' capacity to absorb water and nutrients (Lambert and Bekal 2002). In a 5-month greenhouse experiment, hop plants (cultivar Late Cluster) inoculated with four *H. humuli* cysts had a root weight loss of 68% relative to the noninoculated plants (Sen and Jensen 1969). Aboveground symptoms caused by plant-parasitic nematodes include stunted growth, wilting, leaves yellowing, nutrient deficiencies, and in severe infestations, the death of plants (Jones et al. 2013). It has been reported that the concentration of minerals such as N, P, Mg, and Mn were reduced when hop plants ('Cascade') were inoculated with *H. humuli*, showing severe nutrient deficiency symptoms under greenhouse conditions (Hafez et al. 1999). Hop height and fresh and dry weight of shoots can be also affected. In Australia, Hay and Pethybridge (2003) reported that *H. humuli* infestation caused a reduction of up to 38% in dry hop cultivar Pride of Ringwood per bine. Most recently, Darling et al. (2021) determined under greenhouse conditions that *H. humuli* caused a reduction in bine heights of approximately 40% to young hop cultivar Centennial. Von Mende and McNamara (1995b) found with greenhouse threshold studies that low population densities of *H. humuli* eggs stimulated plant growth: up to 100 eggs/g of soil increased plant height. However, densities higher than 500 eggs/g of soil resulted in bine stunting.

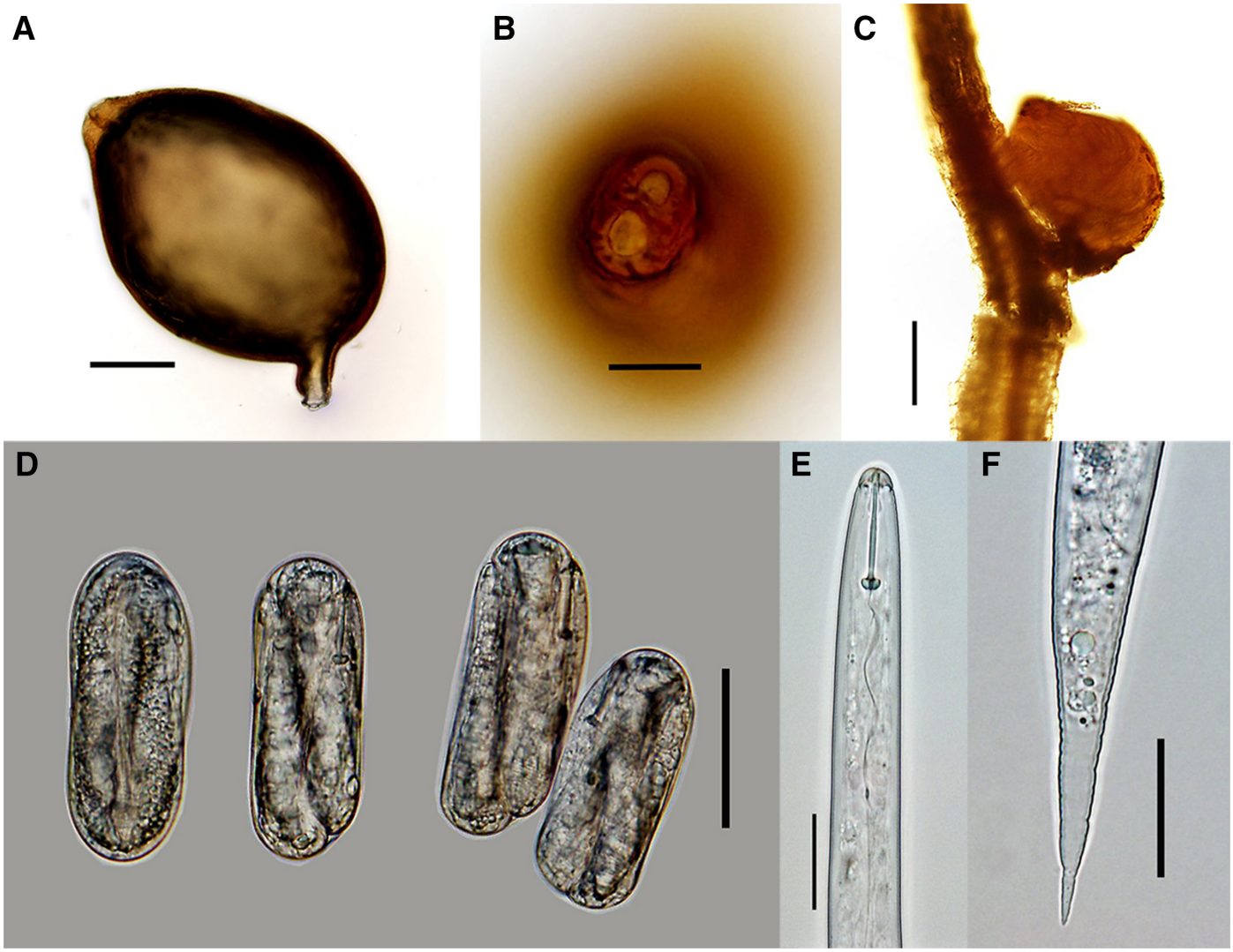

FIGURE 2
Heterodera humuli **A,** cyst with a lemon-shape and prominent vulval cone, **B,** type of fenestration, bifenestrate, and **C,** cyst attached to hop root and filled with eggs, **D,** eggs with J2 inside, **E,** cephalic region and stylet, and **F,** J2 tail. Scales: A and D, 50 µm; B, 100 µm; C, 200 µm; and E and F, 20 µm. Photos by L. Núñez-Rodriguez.

H. humuli Alternate Host Status

Reports on the host status of three prominent plants intertwined with prior *H. humuli* research, *Urtica urens* (annual nettle), *U. dioica* (common nettle), and *Cannabis sativa* (hemp), is conflicting. Before the combination of molecular and morphological strategies to determine differences between related cyst nematodes *H. fici, H. ripae*, and *H. humuli* (and other similar species in the *Humuli* group), these nematodes could be easily confused in early hop surveys due to their overlapping morphology. De Grisse and Gillard (1963) outlined that Sher and Raski (1956) and Nuber (1958) were unable to differentiate *H. fici* from *H. humuli*, and that Cooper (1955) was unable to use perineal patterns to separate these species morphologically. This adds a layer of difficulty to reviewing the host status of these crops for *H. humuli*. Recently, Bernard et al. (2022) described the host status of *H. humuli* on hemp as "contradictory." Some researchers report that *H. humuli* was able to infect and reproduce on hemp (Franklin 1951; Jensen et al. 1962; Sen 1968; Winslow 1954); however, others found that *H. humuli* would not infect hemp (De Grisse and Gillard 1963; Subbotin 1986).

In addition to the hosts discussed above, pea, bean, clover, vetch, mustard, and cucumber were all susceptible to infection by *H. humuli* in greenhouse trials completed by Sen (1968). Hemp, hop, nettle, pea, and vetch all allowed for higher *H. humuli* fecundity, measured as number of cysts, than bean, clover, mustard, and cucumber. *H. humuli*-infested hemp plants had a root weight loss of 63% compared with clean control plants (Sen 1968).

Hop Cyst Nematode Management Strategies

Cyst nematodes (Heteroderidae) are ranked as the second most damaging group of nematodes and are responsible for yield losses worldwide (Jones et al. 2013). Some significant plant-parasitic nematodes in this group include *H. glycines*, *H. schachtii,* and *Globodera* spp., infesting annual crops soybean, sugarbeet, and potato, respectively (Perry et al. 2018). Some cyst nematodes can produce more than 300 eggs and lay dormant in fields for over 20 years without a host and still infect a host; therefore, leaving fields fallow is not an effective form of management (Grainger 1964; Folkertsma et al. 1997). However, a combination of crop rotations with nonhost/poor-hosts, the use of resistant varieties (if available), chemical nematicides, and annual soil sampling can be used to limit yield losses associated with these major plant-parasitic nematodes (Curto 2008; Perry et al. 2018). Unfortunately, many of these strategies are either not applicable to perennial crops, or difficult to apply to trellis systems. For these reasons, developing an effective control method for *H. humuli* could be challenging.

Despite 128 years since the discovery of *H. humuli* in hop yards, there are no effective management strategies available to hop growers experiencing losses by this nematode (Stone and Rowe 1977). Currently, the best way to solve problems caused by *H. humuli* is avoidance and prevention. Performing generic plant hygiene practices such as cleaning machinery between fields, obtaining clean plant material for new yards, and avoiding the transport of infested soil or rootstock is recommended to limit the spread of *H. humuli*;

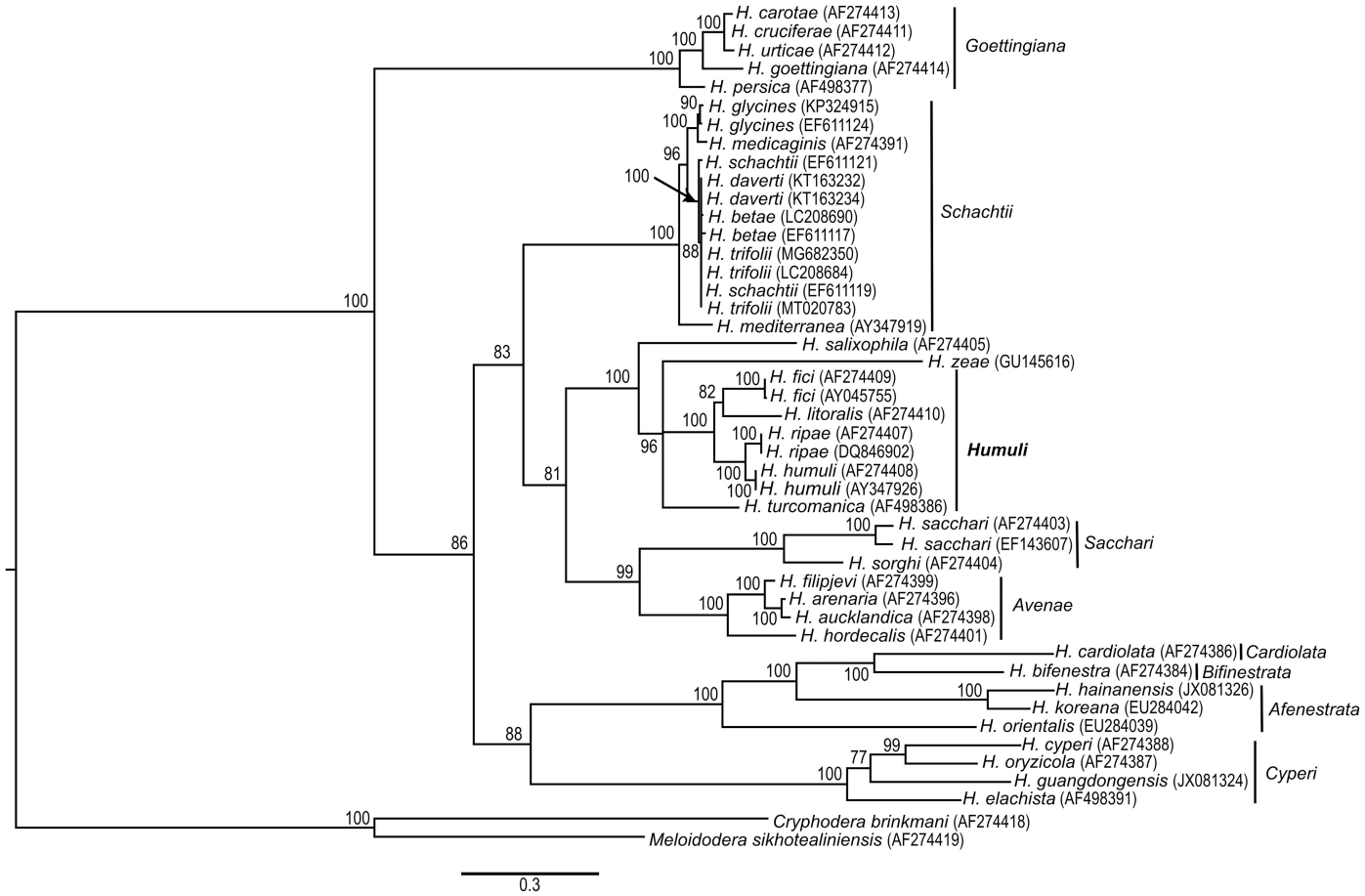

FIGURE 3
Phylogenetic relationships between *Heterodera* species as inferred from Bayesian analysis of the internal transcribed spacer rDNA gene sequences under the GTR + I + G model. Posterior probability values are given for appropriate clades. Group names are given for appropriate species. Sequences were retrieved from GenBank, aligned and trimmed with BioEdit v.7.0.5.3. The best substitution model was determined using jModelTest 2.1.10 v20160303, and the Bayesian analysis was performed using MrBayes v3.2.6. Finally, the tree was visualized using FigTree v1.4.3. Capitalized terms refer to *Heterodera* group names.

however, many studies have revealed that distribution is already very widespread in hop producing regions (De Grisse and Gillard 1963; Stone and Rowe 1977). Hop yards cannot easily be rotated with nonhost/poor-host crops due to trellis system production, which can be a hefty financial investment for the grower.

Currently, registered nematicides for the treatment of plant-parasitic nematodes on hop in the United States are limited to MOCAP EC (Ethoprop; Bayer CropSciences) and Velum Prime (Fluopyram; Bayer CropSciences) (Warner et al. 2015). However, neither of these products have been evaluated for their efficacy against *H. humuli* in published studies. A lack of published efficacy trials leaves no concrete chemical recommendations for viable control of *H. humuli*.

Conclusion

H. humuli was once described as a pest that likely posed a threat to the hop industry, yet lacked documented yield losses and evidence to prompt control. Sen (1968) and a number of nematologists have contributed adequate evidence to suggest that *H. humuli* has been and is currently posing a significant risk to worldwide hop production, and thus, worldwide beer production. In this review, we outlined key points that contribute to the potential of *H. humuli* to be of economic importance: (i) damage: evidence of nutrient deficiencies, plant stunting, reduced root weight, and yield losses to infested plants; (ii) distribution: widespread throughout most major hop-growing regions worldwide; and (iii) management challenges: currently, six hop varieties account for more than 50% of the U.S. hop production. However, there is no consensus on the number of generations that *H. humuli* can complete in a year or how many years it takes for population densities to reach damaging levels, there is no damage threshold for *H. humuli*, and information on the susceptibility of the most common hop varieties to *H. humuli* is lacking. There is also a deficiency of effective management strategies available to hop growers, which is particularly problematic because traditional cyst management strategies (i.e., crop rotation, resistant varieties) are not an option. Hop growers assume a large financial commitment when installing trellis systems, costing an estimated of $35,759/ha under drip irrigation in the PNW (Hop Growers of America 2022). These pricy trellis systems make abandoning established hop yards unfeasible and costly to hop growers. For these primary reasons, studies on the susceptibility of hop to *H. humuli* and effective methods of control for *H. humuli* are urgently needed and would be useful to hop growers worldwide.

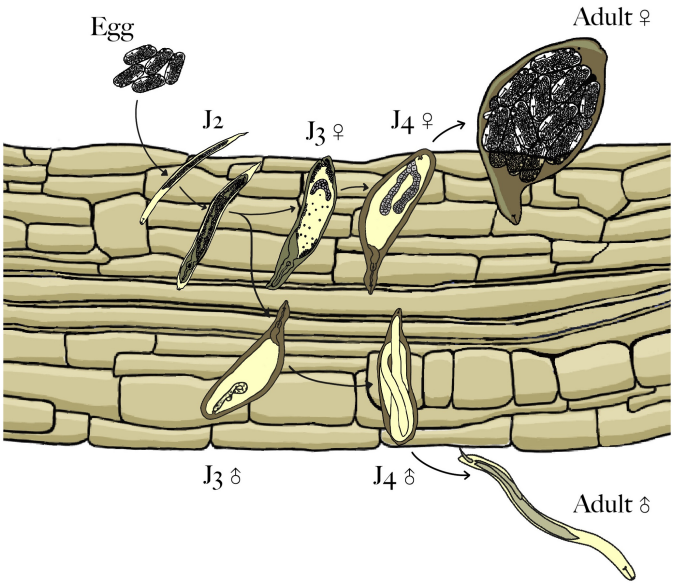

FIGURE 4
Life cycle illustration of *Heterodera humuli*, recreated by the authors and inspired by prior illustrations of the hop cyst nematode life stages (Sen 1968; Stone and Rowe 1977). Drawn by E. Darling.

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