

1 Supplementary information for “Testing the efficacy of different molecular tools
2 for parasite conservation genetics: a case study using horsehair worms (Phylum
3 Nematomorpha)”

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11 **Brief introduction of horsehair worms**

12 Horsehair worms (phylum Nematomorpha) have interesting characteristics from both
13 conservation and ecological perspectives. All the taxa in this phylum are parasites with a
14 complex life cycle, which includes a parasitic infection in the arthropod final host and a free-
15 living adult stage (Schmidt-Rhaesa, 2012; Bolek *et al.*, 2015). Such life cycle makes
16 Nematomorpha one of the few phyla in which all the known species are parasitic (Bolek *et al.*,
17 2015). The most speciose group, which includes worms called “gordiids” (Gordioida; but see
18 Schmidt-Rhaesa 2012), is notoriously known for manipulating their final hosts to jump into
19 water, where the adult worms are released (Bolek *et al.*, 2015; but see Schmidt-Rhaesa 2012,
20 Chiu *et al.* 2020 and Anaya *et al.* 2021 for alternative lifecycles). They can impact food
21 networks in a community by making their hosts jump into water, which makes said hosts easy
22 prey to their predators (Sato *et al.*, 2012). Although common in suitable environments
23 (Schmidt-Rhaesa, 2012; Chiu, 2017), it is known that pollution and human-made changes (e.g.,
24 clear-cut logging and stream remediation) can have negative effects on hairworms, even
25 causing local extinction in some cases (Sato *et al.*, 2014; Chiu *et al.*, 2016; Achiorno *et al.*,

26 2018). Hairworms are also known to be one of the less studied animal group, especially from
27 a molecular perspective (Bolek *et al.*, 2015; Tobias *et al.*, 2017).

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29 **Introduction of the analysed Taiwanese horsehair species**

30 *Chordodes formosanus* is regarded as the most common Nematomorpha species in Taiwan. It
31 is also the only hairworm found both in all the main island of Taiwan and Lyudao so far (Chiu
32 *et al.*, 2011; Chiu, 2017). It is also present in Japan (Chiu *et al.*, 2011). It is known to parasitize
33 three different mantis (order Mantodea) species as definitive hosts; the Indochina mantis
34 *Hierodula patellifera*, the Taiwanese giant mantis *Titanodula formosana* (which is regarded as
35 the main host in Taiwan and it was formerly regarded as a *Hierodula* species: Chiu *et al.*, 2016,
36 2017; Vermeersch, 2020) and the Japanese boxing mantis *Acromantis japonica*. However, it is
37 only reported in *H. patellifera* in Japan and has been sporadically reported in katydids (family
38 Tettigoniidae) species too (Chiu *et al.*, 2011, 2017). Its adult emergence peak in Taiwan is from
39 early June to late August (Chiu *et al.*, 2016, 2017; Chiu, 2017), although it was reported to
40 emerge in Japan around two months later together with *H. patellifera* and it has been
41 sporadically collected in other months in Taiwan too (Chiu *et al.*, 2011; this study). It is usually
42 found during the day inside its definitive hosts (Chiu *et al.*, 2011, 2016) or even road killed
43 with the mantises it parasitises (Chiu, 2017). From what concerns the paratenic hosts, it is
44 known from several non-biting midges (family Chironomidae), the caddisfly *Chimarra*
45 *formosana* and stoneflies in the genus *Kamimuria* (Chiu *et al.*, 2016; Chiu, 2017). The dispersal
46 ability such hosts in Taiwan is not known and flying ability in their clades seems to be species-
47 specific (Ferrington, 2008; Arce *et al.*, 2021). It can also infect the “dead-end hosts” freshwater
48 snails of the genus *Physa* (Chiu, 2017), as other hairworms at the larval stage (see Bolek *et al.*
49 2015).

50 *Acutogordius taiwanensis* is known from Northern to North-Central Taiwan (from

51 Yangmingshan to Taroko National Parks; Chiu, 2017), although it seems to be present in
52 Myanmar too (Chiu *et al.*, 2020). Compared to *C. formosanus*, it is potentially harder to sample.
53 Specifically, its definitive hosts (Ensifera crickets: specifically, at least two species from the
54 raspy cricket family Gryllacrididae and at least 9 different katydid taxa; Chiu *et al.*, 2017) are
55 nocturnal and have a cryptic lifestyle: raspy crickets tend to burrow inside the soil and have an
56 inconspicuous brown colouration (Rentz and John, 1990), while katydids are known for their
57 leaf-like external morphology (Mugleston *et al.*, 2016). Additionally, the infection rate and
58 the population size seem to be lower in *A. taiwanensis* than in *C. formosanus*, which roughly
59 occupy the same environments (M. C. Chiu, pers. comm.). Therefore, it is possible that such
60 species went unsampled in most of its actual range or even extinct in some areas, given that it
61 seems to be very sensitive to human activities (Chiu, 2017). From what concerns the paratenic
62 hosts, the range is very similar to *C. formosanus*: in fact, it has been found inside several
63 Chironomidae midges, in the genus *Kamimuria* and in *Chimarra formosana*. In addition, it can
64 also infect mayflies in the genus *Paraleptophlebia*, beside finishing inside *Physa* snails too
65 (Chiu *et al.*, 2016; Chiu, 2017). As with *C. formosanus*, the dispersal ability of the paratenic
66 hosts is not known, while some of the definitive hosts are present in other countries too (e.g.,
67 *Holochlora japonica*: Cigliano *et al.*, 2023) and they therefore should be able to disperse. Given
68 this, dispersal by hosts may be a possibility. Furthermore, given its relatively big host range, it
69 is possible other definitive hosts are used but they are not reported yet. It can be usually seen
70 inside its definitive hosts from mid-May to late October (Chiu, 2017), although it has been
71 sporadically reported in November too (Chiu *et al.*, 2017).

72 *Gordius chiashanus* is, at the time of the writing of this article, the last described
73 Taiwanese hairworm species and it is reported from mid altitude areas (1100-1700 m a.s.l.) in
74 Taiwan (Chiu *et al.*, 2020). It is one of the only two known Nematomorpha species to be present
75 in wet soils at the adult stage, the other one being *Gordius terrestris* in the United States (Anaya

76 *et al.*, 2021). However, *G. chiashanus* seems to be able to breed in both water and wet soils
77 (Chiu, 2017; Chiu *et al.*, 2020), while *G. terrestris* breeds inside the soil (Anaya *et al.*, 2021).
78 Potential terrestrial paratenic hosts of *G. chiashanus* are not currently known, while it has been
79 sporadically found inside the mayfly *Ephemera orientalis* (Chiu *et al.*, 2020). The only known
80 definitive host is a species from the millipede genus *Spirobolus* (Chiu *et al.*, 2020). Although
81 sampling the adult with the definitive hosts can be challenging (Chiu, 2017), free-living *G.*
82 *chiashanus* adults tend to aggregate together during breeding season, which allows the
83 collection of several individuals at the same time (Chiu *et al.*, 2020).

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85 **References**

86 **Achiorno, CL, de Villalobos, C, and Ferrari, L** (2018). Susceptibility of *Chordodes nobilii*
87 (Gordiida, Nematomorpha) to three pesticides: Influence of the water used for dilution on
88 endpoints in an ecotoxicity bioassay. *Environmental Pollution* **242**, 1427-1435. doi:
89 10.1016/j.envpol.2018.08.006.

90 **Anaya, C, Hanelt, B, and Bolek, MG** (2021). Field and laboratory observations on the life
91 history of *Gordius terrestris* (phylum Nematomorpha), a terrestrial nematomorph. *The*
92 *Journal of Parasitology* **107**(1), 48-58. doi: 10.1645/20-53.

93 **Arce, AP, Hörren, T, Schletterer, M, and Kail, J** (2021). How far can EPTs fly? A
94 comparison of empirical flying distances of riverine invertebrates and existing dispersal
95 metrics. *Ecological Indicators* **125**, 107465. doi: 10.1016/j.ecolind.2021.107465.

96 **Bolek, MG, Schmidt-Rhaesa, A, De Villalobos, LC, and Hanelt, B** (2015). Phylum
97 Nematomorpha. In Thorp J, Rogers DC (eds). *Thorp and Covich's Freshwater*
98 *Invertebrates*: Academic Press, pp. 303-326. doi: [https://doi.org/10.1016/B978-0-12-](https://doi.org/10.1016/B978-0-12-385026-3.00015-2)
99 [385026-3.00015-2](https://doi.org/10.1016/B978-0-12-385026-3.00015-2).

100 **Chiu, MC** (2017). *Biodiversity of the Taiwanese horsehair worms and the host morphological*

101 *development manipulated by infection*. PhD thesis, National Taiwan University, Taipei,
102 Taiwan. doi: <https://doi.org/10.6342/NTU201700507>.

103 **Chiu, MC, Huang, CG, Wu, WJ, and Shiao, SF** (2011). A new horsehair worm, *Chordodes*
104 *formosanus* sp. n.(Nematomorpha, Gordiida) from *Hierodula* mantids of Taiwan and
105 Japan with redescription of a closely related species, *Chordodes japonensis*. *ZooKeys* **160**,
106 1-22. doi: 10.3897/zookeys.160.2290.

107 **Chiu, MC, Huang, CG., Wu, WJ., and Shiao, SF** (2016). Annual survey of horsehair worm
108 cysts in northern Taiwan, with notes on a single seasonal infection peak in chironomid
109 larvae (Diptera: Chironomidae). *The Journal of Parasitology* **102**(3), 319-326. doi:
110 10.1645/15-907.

111 **Chiu, MC, Huang, CG, Wu, WJ, and Shiao, SF** (2017). A new orthopteran-parasitizing
112 horsehair worm, *Acutogordius taiwanensis* sp. n., with a redescription of *Chordodes*
113 *formosanus* and novel host records from Taiwan (Nematomorpha, Gordiida). *ZooKeys*
114 **683**, 1-23. doi: 10.3897/zookeys.683.12673.

115 **Chiu, MC, Huang, CG, Wu, WJ, Lin, ZH, Chen, HW, and Shiao, SF** (2020). A new
116 millipede-parasitizing horsehair worm, *Gordius chiashanus* sp. nov., at medium altitudes
117 in Taiwan (Nematomorpha, Gordiida). *ZooKeys* **941**, 25-48. doi:
118 10.3897/zookeys.941.49100.

119 **Cigliano, MM, Braun, H, Eades, DC and Otte, D** (2023). *Holochlora japonica*. *Orthoptera*
120 *Species File*, Version 5.0/5.0.
121 <http://orthoptera.speciesfile.org/Common/basic/Taxa.aspx?TaxonNameID=1138889>
122 (accessed 6 June 2023)

123 **Ferrington, LC** (2008). Global diversity of non-biting midges (Chironomidae; Insecta-
124 Diptera) in freshwater. *Hydrobiologia* **595**, 447–455. doi: 10.1007/s10750-007-9130-1.

125 **Mugleston, J, Naegle, M, Song, H, Bybee, SM, Ingley, S, Suvorov, A, and Whiting, MF**

126 (2016). Reinventing the leaf: multiple origins of leaf-like wings in katydids (Orthoptera:
127 Tettigoniidae). *Invertebrate Systematics* **30**(4), 335-352. doi: 10.1071/IS15055.

128 **Rentz, DCF and John, B** (1989). Studies in Australian Gryllacrididae: taxonomy, biology,
129 ecology and cytology. *Invertebrate Systematics* **3**(8), 1053-1210. doi: 10.1071/IT9891053.

130 **Sato, T, Egusa, T, Fukushima, K, Oda, T, Ohte, N, Tokuchi, N, Watanabe, K, Kanaiwa,**
131 **M, Murakami, I and Lafferty, KD** (2012). Nematomorph parasites indirectly alter the
132 food web and ecosystem function of streams through behavioural manipulation of their
133 cricket hosts. *Ecology Letters* **15**, 786–793. doi: 10.1111/j.1461-0248.2012.01798.x.

134 **Sato, T, Watanabe, K, Fukushima, K, and Tokuchi, N** (2014). Parasites and forest
135 chronosequence: Long-term recovery of nematomorph parasites after clear-cut logging.
136 *Forest Ecology and Management* **314**, 166-171. doi: 10.1016/j.foreco.2013.12.004.

137 **Schmidt-Rhaesa, A** (2012). Nematomorpha. In Schmidt-Rhaesa A (ed.). *Handbook of*
138 *Zoology: Gastrotricha, Cycloneuralia and Gnathifera*: De Gruyter. pp. 29-145. doi:
139 10.1515/9783110272536.29.

140 **Vermeersch, X. H.** (2020). *Titanodula* gen. nov., a new genus of giant Oriental praying
141 mantises (Mantodea: Mantidae: Hierodulinae). *Belgian Journal of Entomology* **100**, 1–18.

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