Text S1. Additional information on the DNA extraction using GF/F filters.

We extracted total DNA on GF/F filters using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the method described in a previous study (Uchii, Doi, and Minamoto, 2016). Briefly, we placed each filter in the suspended part of a Salivette tube (Sarstedt, Numbrecht, Germany), and then added 440 µL of solution, composed of 40 µL Proteinase K and 400 µL Buffer AL, to the filter. After incubation at 56 °C for 30 min, we collected the liquid held on the filter by centrifugation. To increase the yield of eDNA, we rewashed the filter with 220 µL TE buffer for 1 min and collected the liquid again by centrifugation. We added 400 µL ethanol to the collected liquid, and transferred the mixture to a spin column. Subsequently, we followed the manufacturer's instructions, and the total eDNA was eluted in 100 µL Buffer AE. All DNA extracts were stored in a freezer at -20 °C until real-time PCR analysis.

Figure S1. Comparison of sequences between the target species, closely related species, and the species-specific TaqMan probe. The sequence of our designed probe (Hida_12S_P) was different from those of *H. hidamontanus and H. stejnegeri* by 3 nt, and that of *H. yatsui* by 2 nt.

Sequences (5' → 3')

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
H.kimurae	C	C	T	T	A	A	A	C	T	T	T	G	G	A	G	C	C	T	A	C	C	C	G	C	C	T	G
H.hidamontanus	C	C	T	T	A	A	A	C	T	T	T	G	G	A	A	T	C	T	G	C	C	C	G	C	C	T	G
H.naevius	C	C	T	T	A	A	A	C	T	T	T	G	G	A	A	T	C	T	A	C	C	C	G	C	C	T	G
H.yatsui	C	C	T	T	A	A	A	C	T	T	T	G	G	A	A	T	C	C	A	C	C	C	G	C	C	T	G
Hida_12S_P	C	C	T	T	A	A	A	C	T	T	T	G	G	A	G	C	C	T	A	C	C	C	G	C	C	T	G