

The draft assessment report has been prepared by the applicant and is being circulated for public comment in compliance with section 303EF (2b) of the EPBC Act. The views and opinions expressed in the report are those of the applicant and do not necessarily reflect those of the Australian Government or the Minister for the Environment.

The Department has not edited the report. The Department prepared the introduction to the additional information.

Draft Assessment Report and Application to Amend the List of Specimens Taken to be Suitable for Life Import

Section 1:

Taxonomic Information on Hymenolepis diminuta:

- a. Family Name: *Hymenolpididae*
- b. Genus Name: *Hymenolepis*
- c. Species: *Hymenolepis diminuta*
- d. Subspecies: None
- e. Taxonomic Reference: The Atlas of Living Australia;
- f. Common Name: *Hymenolepis diminuta*
- g. The species is endemic to Australia
- h. The specimen is not genetically-modified.

Section 2:

- a. *Hymenolepis diminuta* is not listed as a CITES species - search conducted on both CITES-listed species database hosted by UNEP-WCMC (<https://www.speciesplus.net/>) and the Checklist of CITES species (<http://checklist.cites.org/#/en>)
- b. Conservation Status under the Environment Protection and Biodiversity Conservation Act 1999: The species is not listed as threatened.

Section 3:

Hymenolepis diminuta; are a species of cestode native to Australia according to both *Australian Zoology*; and *Living Australia*; and is, in fact, endemic in all countries with tropical or temperate climate:

The ubiquitous presence of *Hymenolepis diminuta* in Australia is also mentioned throughout the scientific literature including such articles as: *Cestoda from Rattus assimilis* \ *Rodent zoonoses in North Queensland: The Occurrence and Distribution of Zoonotic Infections in North Queensland Rodents*, v; and *A Review of the Helminth Parasites of Australian Rodents*";;_

HDs are highly evolved, and a stable species which has been studied more than any other cestode, partially due to the ease of maintaining the life-cycle in the laboratory. This is a

highly conserved species that has been studied in minute detail, using laboratory and wild sources.^{ix}

As noted above, *Hymenolepis diminuta* is ubiquitous to Australia, as it is native to rats, species of which live in incredibly varied ecologies. According to the *Australian Journal of Zoology*, because two rat species are so widely distributed throughout Australia, they are most commonly used to measure parasite burden. These are *Rattus fuscipes* (the bush rat, which is most commonly found in thickly vegetated moist areas in Queensland, New South Wales, Victoria, South Australia and south-western Western Australia) and *Hydromys chrysogaster* (the Common Water Rat, which is found in marine environments and is widely distributed throughout eastern Australia, but is found as far west as northeastern South Australiaⁱ). *Hymenolepis diminuta* is common to both species. For example, in 1979, Obendorf notes that, "... *Hymenolepis diminuta*, besides being found in bush rats from four Victorian localities (Apollo Bay, Bemm River, Blackwood, and Poweltown) in this present study, has been reported from bush rats in Queensland.'^{xii}

HD has also been found in the less common species, *Rattus sordidus*, which also has wide distribution throughout Australia. "A comparison of worms found in the old endemic, new endemic and the recently arrived cosmopolitan rats indicates that not many species occur in all three groups or even in two of the three groups...Parasite species occurring in all three host groups include the cestode *Hymenolepis diminuta*...."^{xiii} As Smales states: "Species such as the cestode *Hymenolepis diminuta* and the nematode *Heterakis spumosa* may have been introduced or re-introduced by the recent arrival of cosmopolitan *Rattus* species..."^{xiv}

The life cycle of *Hymenolepis diminuta*:

Eggs of *Hymenolepis diminuta* are passed out in the feces of the infected host (rodents). The mature eggs are ingested by an intermediate host (various arthropod adults or larvae, most often Tribolium beetles (mealy worm beetles)) and oncospheres are released from the eggs; these penetrate the intestinal wall of the host and develop into cysticeroid. *Hymenolepis diminuta* infection is acquired by the mammalian host after ingestion of an intermediate host carrying the *Hymenolepis diminuta* cysticeroid (HDC). Humans can be accidentally infected through the ingestion of insects in pre-cooked cereals or other food items, as well as directly from the environment (e.g., via oral exploration of the environment by children). After ingestion, the tissue of the infected arthropod is digested, releasing the larvae in the stomach and small intestine. Maturation of the parasites occurs within 20 days and the adult worms reach an average of around 30 cm in length. Eggs are released in the small intestine and are expelled to the environment in the mammalian host's feces, beginning the cycle again.^{xv}

A search of the scientific research on HDC confirms that there is no literature describing the ability of HDC to act as a vector for other pathogens.

Section 4:

The specimens are not a subspecies. The HOC will be obtained from Biome Restoration Ltd. The HOC are obtained from ova from rats kept at Marshall B&K Universal Ltd. a UK Home Office approved facility which meets or exceeds conditions laid down in the UK Animal Scientific Procedures act code of practice. Biome Restoration Ltd. has been granted a UK Home Office permit for the production of and research into HOC, grown from ova supplied by Marshall B&K. The original source of *Hymenolepis diminuta* was from Dr. William Parker's laboratory, at Duke University in the USA.

Conditions used to maintain the rats ensuring no possible contamination include:

Rats are kept in a negative pressure flexible film isolator, which has HEPA filters on the air inlet and outlets.

The isolator is fumigate it with hydrogen peroxide and ensure there is zero bacterial, viral and fungal contamination in the isolator before population with 2 rats.

Rats will be housed in caging which will exceed the minimum laid down in the UK Animals scientific procedures act code of practice.

A negative pressure isolator containment is used and the rats will be cared for by trained and competent animal technicians.

Their health and welfare will be assessed and documented at least daily using a pre-defined checklist by a trained animal technician. (attached)

The Named Veterinary Surgeon and Named Animal Care and Welfare Officer will be involved in recommending and providing husbandry and veterinary care."

Section 5:

The HOC are for personal use by individuals as a macrobiotic organism, used for biome restoration purposes. That is, they are used similarly to a probiotic organism.

Section 6:

There is no potential environmental impact.

The HOC are treated with high intensity UVC light by Biome Restoration Ltd before dispatch. Repeated attempts to infect rats with UVC irradiated HOC have resulted in either rats with mature HD that do not produce HD ova, or failure to obtain mature HD in the rat population.

Section 7:

As HDs are ubiquitous to Australia, already having established their ecological niche in the native rat populations, there are no possible controls on the species.

^vhttps://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:afd.taxon:4ddaef19-ab2c-4b39-b563-079f84cafd7c#tab_recordsView

^{vi} *Rudo/phi*, C.A. 1819, p.811, Augustus Racker, Bero/ini

^{vii} <http://www.publish.csiro.au/ZO/Z097013>

^{viii} <https://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:afd.taxon:4ddaef19-ab2c-4b39-b563-079f84cafd7c>

^{ix} Arai, H. 1980. *Biology of the Tapeworm Hymenolepis diminuta*. New York: New York Academic Press

^x Sandars, DF. Cestoda from *Rattus assimilis* (Gould, 1858) from Australia. *Journal of Helminthology*. 1957;31 (1-2):65-78.

^{xi} Glazebrook, JS, Campbell, RS, Hutchinson, GW, Stallman, ND. Rodent zoonoses in north Queensland: **the occurrence and distribution of zoonotic infection in North Queensland rodents**. *Australian Journal of Experimental Biology and Medical Science*. 1978. **56**, 147-156).

^{xii} Smales, LR. **A review of the helminth parasites of Australian rodents**. *Australian Journal of Zoology*. 1997. 45(5):505-521.

^{xiii} (Arai, H. 1980. *Biology of the Tapeworm Hymenolepis diminuta*. New York: New York Academic Press.)

^{xiv} (<https://australianmuseum.net.au/bush-rat>) ^{xv} (<https://www.anbg.gov.au/cpbr/WfHC/Hydrornys-chrysogaster/index.html>)

^{xvi} Obendorf, DL. The helminths parasites of *Rattus fuscipes* (Waterhouse) from Victoria, including description of two new nematode species. *Australian Journal of Zoology*. 1979.27 867-79.

^{xvii} (from *Australian Journal of Zoology*):

^{xviii} **Op cit**

^{xix} <https://www.cdc.gov/dpdx/hymenolepiasis/index.html>)

Additional Information

The draft assessment report was reviewed against the Terms of Reference by the Department and a number of issues were identified that needed to be addressed before the document was released for public comment.

The following is the applicant's responses to that request. The numbering corresponds to numbers in the draft assessment report.

1.0: Please provide details of the subspecies or strains proposed to be imported.

Biome Restoration's HDC were sourced from Dr. William Parker's laboratory at Duke University Medical School, in the USA. In turn, Dr. Parker's were sourced from Carolina Biological Supplies in the USA (2700 York Road, Burlington, NC 27215-3398 – www.carolina.com), who get theirs from one ultimate source: Dr. John Oaks' lab at the University of Wisconsin, Madison's, School of Veterinary Medicine.

3.0: Please clarify if the strains or subspecies of *Hymenolepis diminuta* found in Australian rodents is native or introduced. Please provide details of how the Australian strain/s differ from the therapeutic strain/s you propose to import.

There is limited published scientific literature that directly addresses this question. The available literature that is relevant is given below.

In 1990, scientists in the Australian Department of Zoology, Australian National University, did a genetic comparison of their HDC (originally sourced 25 years previously from wild sources (i.e. native) in Australia and ones originally of Canadian origin, and found that there were some differences in worm metabolism between the two strains. However, they concluded that, "...while the biochemical differences were first thought to reflect a difference in genotype, brought about by isolation and subsequent 'bottlenecking' of the gene pool during passage over long periods of time, genetic characterization using alloenzyme electrophoresis *showed the strains to be homozygous and genetically identical at 35 loci.*" [emphasis added] They went on to say that, "Variations in the metabolism of *H. diminuta* may, according to this view, simply be a reflection of the environmental conditions experienced by the parasite, and may not be fixed genetically." That is, the host's individual physiology affects the HD's metabolism and is NOT indicative of a new sub-species of HD.ⁱ

Other studies from elsewhere in the world show similar findings. For example, in 2017, Makki, et. al., studied the HD from grain beetles found in bakeries throughout Iran: "The *H. diminuta* DNA sequences generated in this study were identical and matched 97–100% with similar sequences from GenBank database."ⁱⁱ

When exactly the species was introduced to Australia is unknown but it is now regarded as native as it has been found in Australian rodents since research first began on classifying native Australian wildlife, as per Australian Zoologyⁱⁱⁱ and Living Australia.^{iv}

Due to the lack of scientific literature, Professor Ian Beveridge, Professor of Veterinary Parasitology, University of Melbourne, was contacted for an expert opinion. His answer is given below as a personal communication.

"*H. diminuta* is a common parasite of Australian rodents has been recorded in Australia both from *Rattus rattus* and the native species *R. assimilis* and *R. lutreolus*, with records dating back to at least 1910. (Mackerras, M.J. (1958))^v On this basis, the occurrence of the parasite in *Rattus* spp. indicates that it is already present in both native and introduced species of *Rattus*. Whether it was introduced with *R. rattus* (probably) but was already present in native *Rattus* spp. which arrived on the continent 1 million years ago is unknown at present."^{vi}

Professor Beveridge goes on to say: “Many years ago, the Australian National University imported *H. diminuta* from overseas and maintained it for many years. Unfortunately, that strain has now been lost.”^{vii}

From this, it stands to reason that that laboratory-sourced *H. diminuta* is likely to have already been introduced, and is indistinguishable from native *H. diminuta*.

Please provide details of any differences between the endemic strain/s and the therapeutic strain/s in terms of species they can infect, severity of symptoms and climatic ranges.

Currently, there are no known differences in the species infected, severity of symptoms and climate ranges between various strains of HD.

Please provide details if *Hymenolepis diminuta* are used for any other purpose than human biome restoration or in other species? Attachment A

Hymenolepis diminuta are also commonly used for laboratory research purposes (see PubMed for current research) and are used regularly in ordinary biology classes in grade schools through university level (for example, Carolina Biologic Supplies ships to schools throughout the USA).

Please provide additional details on:

Professor William Parker, of Duke University Medical School, USA, a leading researcher into the specific species, *Hymenolepis diminuta*, was also contacted for further information.

□ All the species that *Hymenolepis diminuta* can infect including intermediate species in the parasites life cycle.

“The Norwegian rat is the only species known to be commonly (frequently) colonized with *Hymenolepis diminuta*. Extensive studies have shown that the door mouse (*Mus musculus*), very closely related to the Norwegian rat, cannot be colonized by *Hymenolepis diminuta*. This suggests high species specificity for the helminth in terms of a primary host. Although some other rodent species such as black rats and hamsters may be colonized, such colonization may be inefficient, uncommon, and/or unreliable. The helminth is not known to (and not expected to) colonize marsupial species. Colonization of dogs and humans is extremely rare. *Hymenolepis diminuta* can colonize any insect species which ingests fecal material from colonized rodents.”^{viii}

□ Life span of the tapeworm in all host species.

“*Hymenolepis diminuta* lives for the lifespan of its hosts, and thus its lifespan is limited only by the lifespan of its primary hosts. This equates to about three years for the adult living in a rat, and for weeks to months for the cysticercoid (larva) living in insects.” Professor William Parker indicates that *H. diminuta*, live for up to 2 weeks in humans so do not reach maturity and produce ova.^{ix}

□ Can the tapeworm complete its life cycle in a single host?

No, *H. diminuta* requires an intermediary insect host as part of its life cycle.^x

□ Details of its reproduction rate for example does it produce eggs all year around or seasonally, how many eggs per cycle?

There is no definitive answer to this in the scientific literature. Ova production is likely to be affected by host diet and helminth burden in the host. As stated above, *H. diminuta* in humans are not expected to live long enough to produce ova.

According to the University of Michigan’s Museum of Zoology, “Once the adult *H. diminuta* is embedded in the host, it can produce over 250,000 eggs per day.... There is an extremely low chance for each egg to reach reproductive maturity and that is why *H. diminuta* lays so many eggs.”^{xi}

□ Viability of eggs shed from humans including if commercial sewage treatment kills or attenuates the eggs.

According to Professor Parker: “The eggs are not very stable to start with, and it’s difficult to

imagine that the conditions in a sewage treatment facility would extend their life. But we really can't say anything with confidence unless we conduct some experiments to mimic the conditions of sewage treatment facilities. If they do make it into the sewage treatment facilities, insects won't have the ability to eat them. Thus, it will be a dead end in their life cycle." Professor Parker goes on to point out that that there are many rats currently defecating eggs into existing sewage treatment facilities worldwide, and this is not an issue environmental issue in any country.^{xii}

□ How long viable eggs can survive in faeces.

According to Professor William Parker, "Eggs are seldom shed from humans, and then only under very exceptional and rare circumstances. Eggs are typically viable in the laboratory for approximately 10 days, although a few eggs may survive for over a month. Importantly, eggs, if excreted from humans, are not expected to survive any longer than will eggs excreted from native rats."^{xiii}

Please provide details of screening of the therapeutic product you are proposing to import in terms of potential bacterial, viral, protozoa, fungal, other parasites etc. in the carrier fluid.

Currently, Biome Restoration Ltd. is the only commercial supplier shipping HDC internationally. Their HDC is sourced from ova produced in rats kept under strict isolation in a UK Home Office approved and licenced facility. The HDC are maintained in a shipping fluid that is filtered immediately before use through 0.2 µm filters and each vial is exposed to 110 W of UVC light immediately before closure. Biome Restoration regularly ships to academic laboratories, and they, in turn, regularly ship to each other for research purposes.

Although the full disease risk will need to be assessed by the Department of Agriculture and Water Resources, the Department of the Environment and Energy needs to consider potential pathogen risks to native species as part of its assessment.

Hymenolepis diminuta is not considered a parasite or a pathogen. In its native host, laboratory studies have shown that it is benign and protective from dangerous inflammatory responses.^{xiv}

4.0: Please list all suppliers of therapeutic *Hymenolepis diminuta*

Biome Restoration Ltd. – Unit 8-4-8 Harpers Mill, White Cross Business Park, Lancaster, UK LA1 4XF, +44 01524 555 982 – www.biomerestoration.com

6.0: In light of the questions and additional information requested above, please reconsider if there may be any potential environmental impacts.

As noted above, *Hymenolepis diminuta* is:

1. A species already native to Australia
2. Endemic to all regions of Australia.
3. Commonly found in rodents (mainly rats) throughout Australia.
4. If released into the wild, imported HD poses no meaningful threat, as those species that can be colonized by it already are.
5. Current research shows that there are no meaningful differences in strains of HD, as genetic studies show great homogeneity.
6. Thus, it can only be concluded that there is no meaningful environmental impact from importing HDC into the country. Again, Professor Ian Beveridge has kindly offered to give an expert opinion

7.0: Please provide details of any state or territory controls or legislation relating to *Hymenolepis diminuta*. Is it listed as a notifiable disease or parasite to any species?

There are no territory regulations or legislation relating to HD.

According to Professor Parker, infection with HD in its native host or humans, is not viewed as a notifiable disease in any country. *Hymenolepis diminuta* is not considered a parasite or a pathogen. In its native host, laboratory studies have shown that it is benign and protective from dangerous inflammatory responses.^{xv} Hymenolepiasis is a recognised but non-notifiable disease, although the primary cause of this is a different species, *Hymenolepis nana*, the dwarf rat tapeworm.

□ Is the use of *Hymenolepis diminuta* treatment subject to any regulation by health agencies?

HD is not regulated as a medicine or healthcare product in either Australia or the UK.

□ Is *Hymenolepis diminuta* treatment approved by agencies such as the Therapeutic Goods Administration?

To the best of my knowledge the use of helminths has not been reviewed by the Therapeutic Goods Administration.

□ Can *Hymenolepis diminuta* be imported into Australia under the *Biosecurity Act 2015* administered by the Department of Agriculture and Water Resources?

Hymenolepis diminuta is not listed as a banned species for import by the Biosecurity Act of 2015 and does not appear as a regulated species.^{xvi}

ⁱ Bennet, EM, Behm, CA, Bryant, C. The role of the host in the regulation of end-product formation in two strains of the rat tapeworm, *Hymenolepis diminuta*. *International Journal for Parasitology*. 1990;20(7):841-848.

ⁱⁱ Makki, MS, et. al. Identification of *Hymenolepis diminuta* cysticeroid larva in *Tribolium castaneum* (Coleoptera: Tenebrionidae) beetles from Iran. *Journal of Arthropod-Borne Diseases*. 2017;11(2):338-343.

ⁱⁱⁱ <http://www.publish.csiro.au/ZO/ZO97013>

^{iv} <https://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:afd.taxon:4ddaef19-ab2c-4b39-b563-079f84cafd7c#sequences>

^v Mackerras, M.J. (1958) Catalogue of Australian mammals and their recorded internal parasites. *Proceedings of the Linnean Society of New South Wales*, 83, 126.

^{vi} Personal Communication with Dr. Ian Beveridge, August 2018

^{vii} Personal communication with Dr. Ian Beveridge, August 2018

^{viii} Personal communication with Dr. William Parker, August 2018

^{ix} Personal communication with Dr. William Parker, August 2018

^x Arai, HP. *Biology of the Tapeworm Hymenolepis diminuta*. Sydney: Academic Press. 1980.

^{xi} https://animaldiversity.org/accounts/Hymenolepis_diminuta/

^{xii} Personal communication with Dr. William Parker, August 2018

^{xiii} Personal communication with Dr. William Parker, August 2018

^{xiv} Personal communication with Dr. William Parker, August 2018

^{xv} Personal communication with Dr. William Parker, August 2018

^{xvi} <http://www.agriculture.gov.au/import/goods/live-animals>