The genus *Raffaelea* was named in 1965 by von Arx and Hennebert and amended two years later by Batra (1967) for ambrosia fungi that create sporodochia (small compact cushion-shaped masses of somatic hyphae covered with conidiophores, Fig. 2). Members of the genus produce terminal conidia acropetally (youngest conidium is at the tip or distal end of the chain) (Ulloa and Hanlin 2000) and sympodially (conidia are produced on a conidiophore that terminates and branches repeatedly, producing a zigzag pattern) (Ulloa and Hanlin 2000). In fungal taxonomy, each species is typified by one specimen (a collection, a culture – dried or otherwise preserved in a metabolically inactive state, microscope slide mount) (Alexopoulus et al. 1996), and a "type species" is the basis for description of a genus. *Raffaelea ambrosiae* is the type species for *Raffaelea* (von Arx and Hennebert 1965).
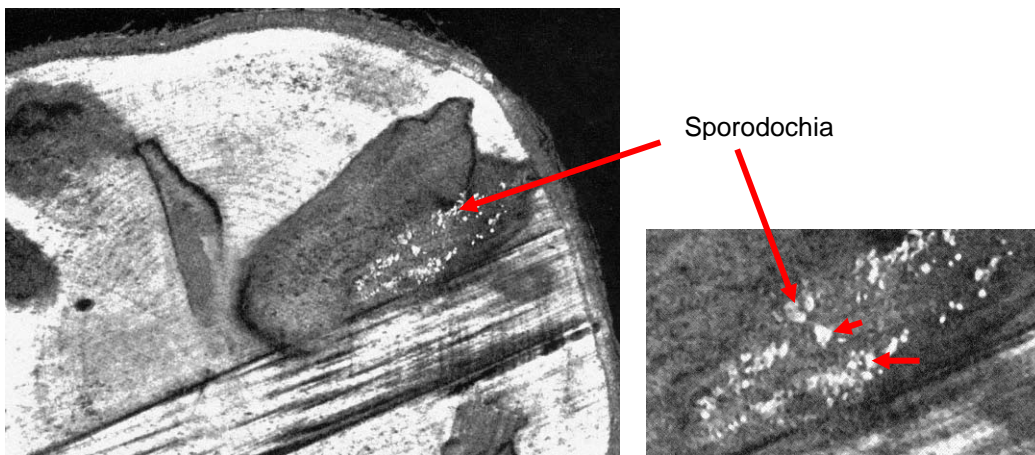

Fig. 2. Arrows point to sporodochia (small white pustules) growing on a cross section of a maple.
[Reproduced from Batra (1967).]

A sexual stage of *Raffaelea* has not been found. Based on numerous similarities to other asexual forms of fungi for which the sexual form is known, *Raffaelea* is classified in the Ascomycetes (Kirk et al. 2001). The morphology and growth habit of spore-producing hyphae (called conidiomata); the shape, septation and color of spores; and the processes involved in conidial production are used to classify asexual fungi (Kirk et al. 2001). Molecular tools provide other evidence of taxonomic identity. Results of a cladistic analysis of 18S ribosomal DNA sequences showed that seven of eight species of *Raffaelea* appear to be phylogenetically related to the genus *Ophiostoma* (Jones and Blackwell 1998). This work occurred prior to the description of *R. quercivora*.

Formal description of the genus *Raffaelea* and type species *R. ambrosiae.*
"Sporodochia superficial, effuse, confluent (coalescent), fertile, lush. Conidiophores in fascicles, straight, septate, with a thick base that tapers toward the apex. Conidia originating in succession from the apex of the conidiophore, obovoid, with a conical truncated base, single-celled, hyaline. Aleuriospore solitary, arising from the apex of a septate hypha, globose to even turbinate, single-celled, hyaline, thick covering. *Raffaelea ambrosiae* is the type species,

found in *Platypus cylindrus* FAB. in *Quercus*, in England. Type culture in dried agar agaro dessicata CBS 185.64est" (Translated from von Arx and Hennebert (1965)).
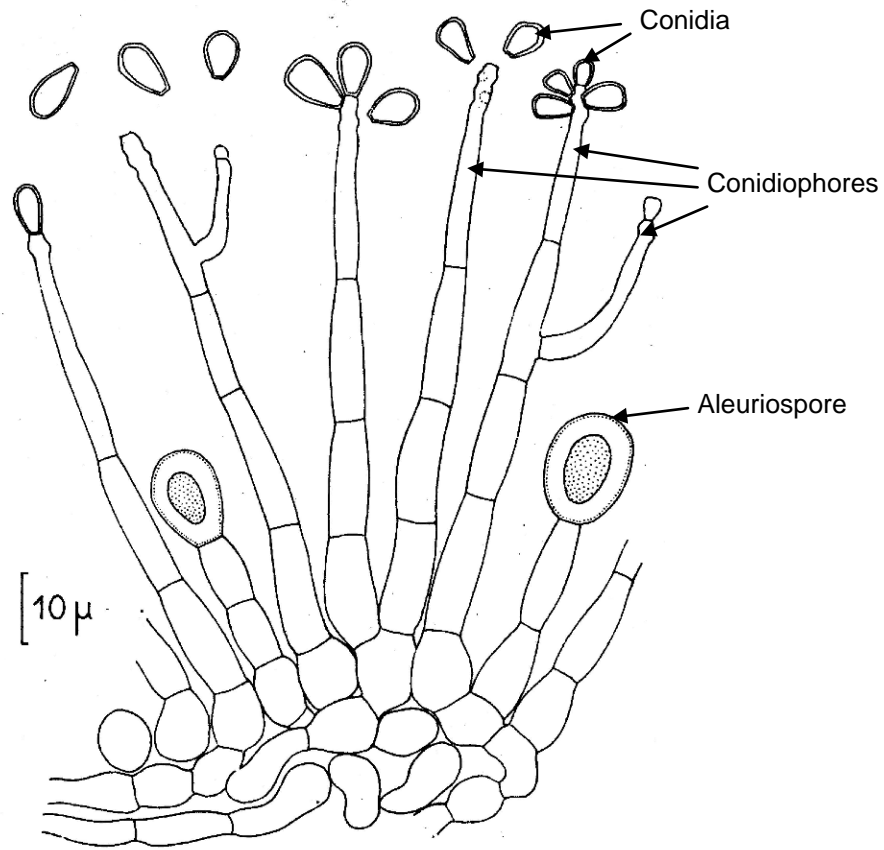
Fig. 3. "*Raffaelea ambrosia*. Conidiophores with conidia and aleuriospores"
[Reproduced from von Arx and Hennebert (1965).]

The original description provided by von Arx and Hennebert was based on examination with a light microscope. In a recent examination of *R. ambrosia* conidial development using scanning electron microscopy, Gebhardt and Oberwinkler (2005) report annellidic percurrent proliferation rather than sympodial production.

Raffaelea quercivora. "Colonies on [potato dextrose agar] PDA at 20–25°C [68-77°F] effuse, spreading rapidly, reaching 80 mm [~3 $^1/_8$ in] diameter in 5 days with an indistinct white margin, appearing water-soaked and mucilaginous; aerial mycelium abundant, floccose, composed of branched, septate, hyaline, smooth hyphae, arranged in fascicles and simulating coremia, reaching 1 cm high; color diffusing and turning pale olive to brown-olive after 2 weeks; odor fragrant, resembling that of ethyl alcohol (Fig. 4). Sporodochia of several fascicles, becoming confluent and mucilaginous. Conidiophores macronematous, mononematous, formed in sporodochia or produced separately, simple or branched, straight, hyaline, septate, smooth, 16.5–22.5 x 0.9–1.5µm (Fig. 5). Conidiogenous cells gradually narrowed toward the apex, polyblastic, sympodial,

indeterminate, discrete or integrated, terminal, hyaline, smooth, with a series of flat, scarcely protruding scars situated toward the apex (Figs. 6B and 7C). Conidia short-clavate slimy, borne in acropetal order, hyaline, aseptate, straight, smooth, eguttulate, obovoid to pyriform, tapered markedly toward the truncate base, apex obtuse, frequently yielding sprout cells, formed in droplets, 3.1–4.7 x 2.0–2.4µm" (Figs. 6A and 6C, 7A and 7B)" (Kubono and Ito 2002). Aleuriospores were not described for this species.
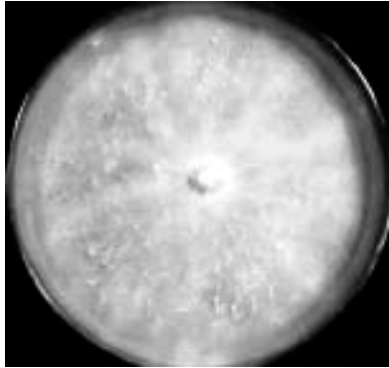

Fig. 4. "*Raffaelea quercivora* colony with sporulation on potato dextrose agar (PDA) at 20⁰ C after 10 days."
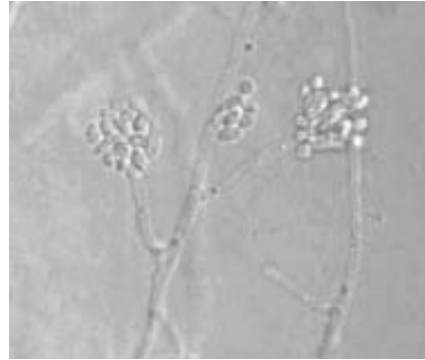[Photo and legend from Kubono and Ito (2002)].

Fig. 5. "*Raffaelea quercivora* conidiophores and conidia on PDA."
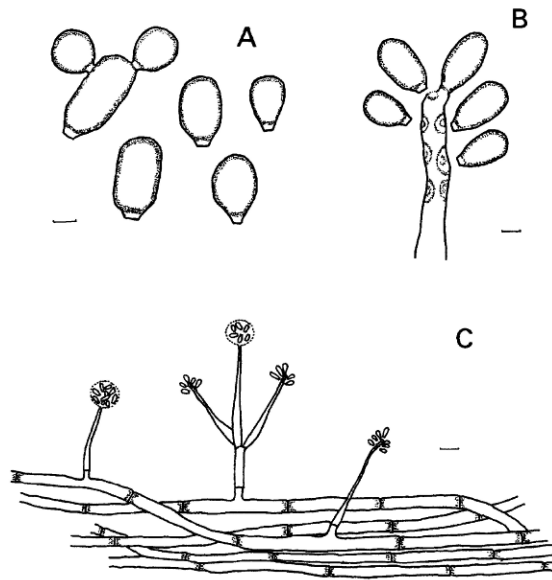[Photo and legend from Kubono and Ito (2002).]

Fig. 6. "*Raffaelea quercivora*. A. Conidia. B. Conidiogenous cell with holoblastic proliferation and cicatricial scars. C. Conidia and conidiophores; one at the left and one at the center showing conidia in droplet. Bars A, B are 1 µm, C is 5 µm."
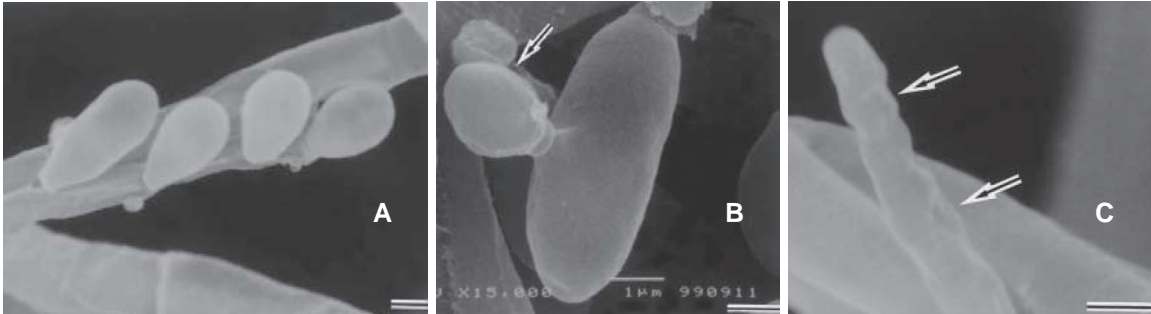[Drawings and legend from Kubono and Ito (2002).]

Fig. 7. "*Raffaelea quercivora* on PDA observed by SEM. A. Conidiogenous cell with holoblastic proliferation and conidia. Bar 1µm. B. Conidia and sprout cell (*arrow*) produced from conidia. Bar 1µm. C. Conidiogenous cell with a series of flat cicatricial scars (*arrows*). Bar 10 1µm."
[Photos and legend from Kubono and Ito (2002).]

Biology and Ecology

Raffaelea quercivora is one of many species of ambrosia fungi, so-called because of their symbiotic relationships with some species of wood boring Scolytidae and Platypodidae. Most often associated with recently logged timber and trees of low vitality, ambrosia fungi line the tunnels and galleries created in the sapwood and heartwood by their associated insects with a continuous layer of hyphae and conidia-bearing conidiophores (Batra 1967). The insect relies on the fungus for nourishment during at least some its life stages, meanwhile providing the fungus with protection and means of dispersion and inoculation (Baker 1963, Kinuura 2002).

Similar to other ambrosia fungi, *R. quercivora* has not been found apart from the insect, *Platypus quercivorus* (Coleoptera: Platypodidae). Thus, the life cycle of the fungus in nature is intimately related to the life cycle of this insect (Fig. 8). The reciprocal is not necessarily true. Conidia of *R. quercivora* are carried by *P. quercivorus* and contact the plant host when the insect bores into the sapwood and heartwood of the tree (Kinuura 2002). Conidia may germinate directly, or produce sprout cells which germinate and elongate into septate hyphae (Fig. 8A) (Kubono and Ito 2002). Hyphae grow in the tunnels and galleries excavated by the beetle (Fig. 8B), and eventually line the entire surface (Kinuura 2002). Hyphae may aggregate into bundles, called fascicles (Fig. 8C), which may further aggregate into sporodochia (see 'Pest Description') from which conidiophores develop (Fig. 8D) and produce conidia (Fig. 8E). While providing nourishment for the insect, the fungus continues to grow, and hyphae extend into the wood adjacent to the tunnel.

The creation of insect galleries and the presence of the fungus stimulate defense responses from the tree, which include the production of tyloses (parenchyma cell overgrowths) that extend into the xylem and prevent the ascent of water (Yamada and Ichihara 2003). In the meantime, the female insect lays eggs, which hatch in about a week. Larvae feed on the fungus until they pupate. Young adults may emerge in autumn (October, November), allowing for the possibility of a second generation that year, or remain in the tree over the winter and emerge

in the spring (Sone et al. 1998). Young adults consume and acquire the fungus in their mycangia. When they emerge from the tree, they carry the fungus. The insects move on to another tree and bring the pathogen to a new host. The fungus may go through many life cycles from the time it is introduced into the tree until the time the next generation of insects emerges with the fungus.
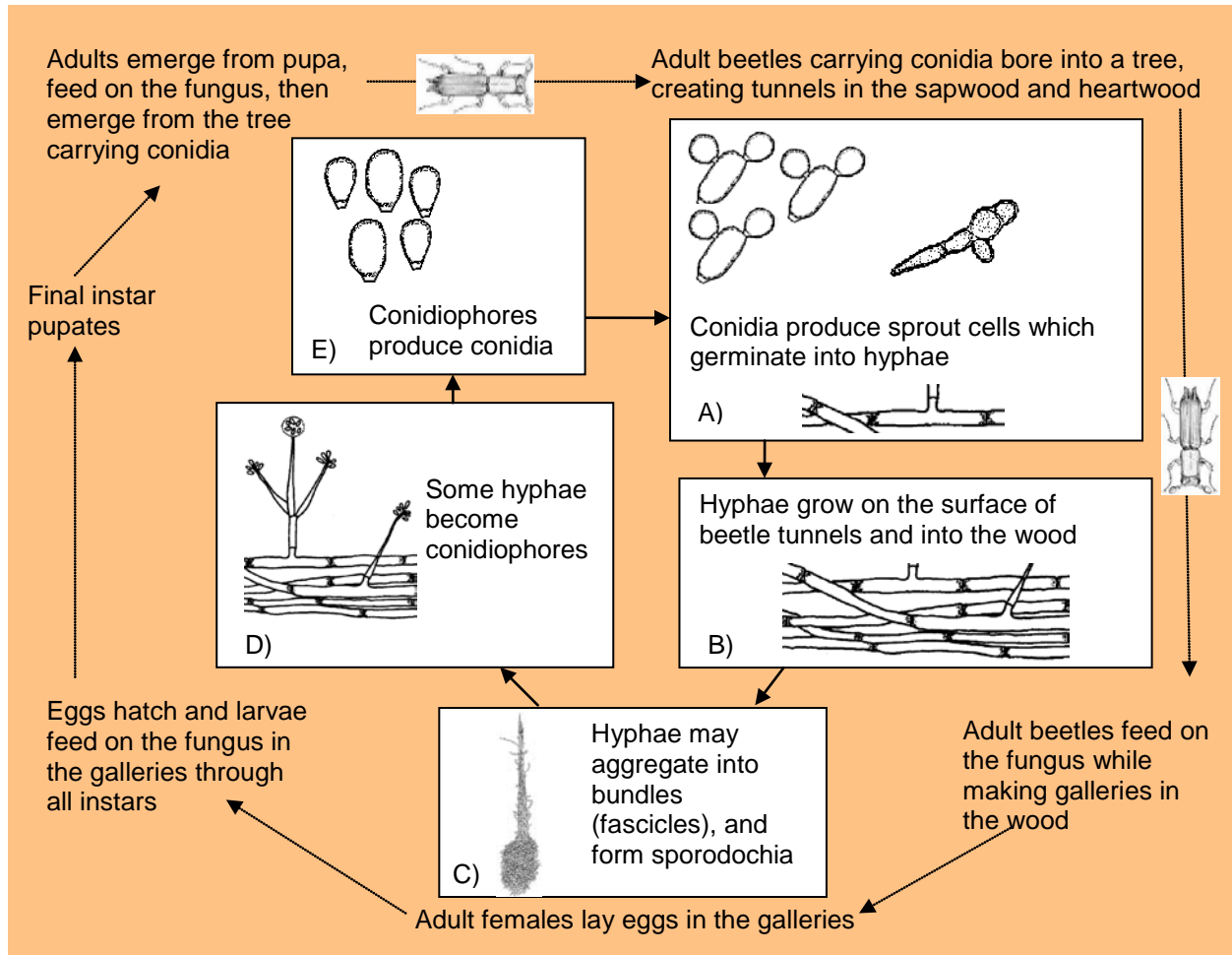
Fig. 8. Life cycle of *Raffaelea quercivora* and its relationship to the vector, *Platypus quercivorus* (images not to scale).
[Drawings from Kubono and Ito (2002), Batra (1967), and Ebeling (1975).]

Infection by the fungus is presumed to occur shortly after initial attack by *P. quercivorus,* when the fungus is first introduced into the host. Attacks start in May or June and may extend through August (Mori et al. 1995, Saito et al. 2001). Following attack by *P. quercivorus*, trees discolor and wilt within 2-3 months and die that first season or by the following spring (Kubono and Ito 2002, Kobayashi and Ueda 2003). Insect infestations of standing trees and logs were highest at the beginning of the season (June - early July) (Mori et al. 1995, Sone et al. 1998).

While there is a tendency to attribute oak mortality to *R. quercivora* when *P. quercivorus* is present, in many cases the fungus was not actually isolated and identified. Spread of the disease within a stand appears to be a function of vector behaviors and patterns. Disease initially occurs at the edge of a gap or forest and on upper slopes; spread among trees occurs outward and downward from an infection epicenter (Kamata et al. 2002, Esaki et al. 2004). Little is known about the likelihood of the fungus being dispersed in the movement of infected logs. Symptoms spread faster in stands that have a higher percentage of susceptible hosts (Kamata et al. 2002).

Specific requirements for the growth of the fungus are not known. In general, nutritional quality of the substrate may determine the primary growth form of *R. quercivora* (e.g. conidia versus hyphae) (Unknown 2002). *Raffaelea quercivora* will grow in culture on PDA at 20-25°C [68-77°F], and produce all of its life stages (Kubono and Ito 2002). In logs, the water content of the wood impacts the growth of *R. quercivora* (Kobayashi et al. 2004).

Hyphae, the filamentous body of the fungus, occur in the tunnels and galleries created by *P. quercivorus*, and will grow into the ray and parenchyma cells of the heartwood. In the process, the fungus stains the wood (Fig. 9). Fragments of hyphae also probably occur in the mycangia and on the outer body surface of the insect vector. On PDA, *R. quercivora* grew to an 80 mm [~3 $^1/_8$ in] diameter colony in five days at 20-25°C [68-77°F] (Kubono and Ito 2002). The fungus has an odor and color on PDA that are distinct from other fungi and yeasts found in *P. quercivorus* mycangia Kinuura (2002). *Raffaelea quercivora* may be tolerant of temperature extremes depending on humidity levels. Hyphae of a related species - *R. ambrosiae* - died after 30 minutes at 50°C [122°F] and 100% relative humidity (RH) but survived 30 minutes of exposure to 75°C [167°F] at 10-20% RH; hyphae continued to grow after 50 days at 2°C [36°F] (Kaarik 1974). Until further research is conducted we can only suggest that tolerances for *R. quercivora* may be similar. For ambrosial symbionts in general, conidia within mycangia are protected by lipids produced by mycangial glands (Baker 1963).
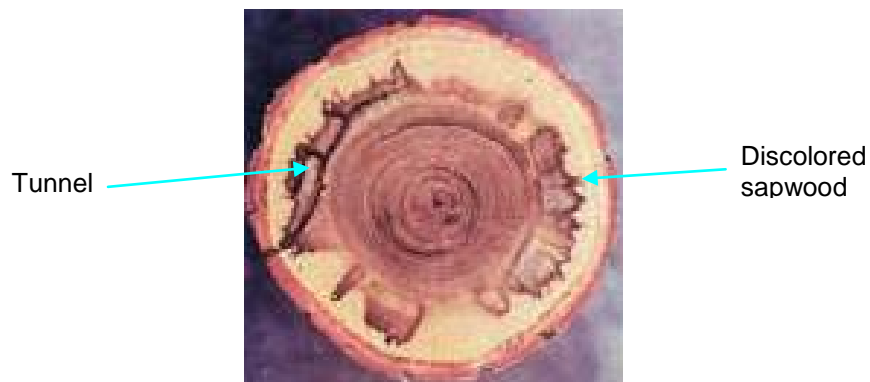

Fig. 9. Cross-section of wilting *Q. mongolica* var. *grosserrata* showing
discolored sapwood and insect tunnels.
[Photo from Ito et al. (2003b).]

Wilting results from disruption of water flow at sites of infection (Kuroda et al. 2002, Yamada et al. 2002, Kuroda et al. 2004). Young hyphae of *R. quercivora* invade the living ray parenchyma cells, and tyloses form in the vessels around the hyphae (Kuroda 2001). Tyloses prevent the flow of water and may also form in response to mechanical wounding (Yamada et al. 2002). Trees with significant blockage die, and variation in the amount of blockage may account for differences in mortality rates among and within species (Kamata et al. 2002).

Pest Importance

The complex of *R. quercivora* and *P. quercivorus* has been associated with the mortality of large numbers of oak trees in Japan. Oaks (*Quercus* spp.), mainly *Quercus serrata* and *Q. mongolica* var. *grosseserrata*, are particularly susceptible (Ito et al. 2003b). Since 1980, 100,000 - 200,000 fagaceous trees have been killed annually (Ito et al. 2003b, Ito et al. 2003a). The extensive oak mortality in Japan may have impacted habitat for Asian black bears, causing them to move into more populated areas (Yamazaki 2004). This resulted in numerous human injuries and the killing of numerous bears to stop bear attacks. Approximately 170 bears were killed in one district alone (Yamazaki 2004).

Raffaelea is among the many genera of ambrosia fungi occurring in symbiotic relationships with a group of wood boring scolytid and platypodid ambrosia beetles, but *R. quercivora* is the first ambrosia fungus that has been reported to kill healthy trees (Kamata et al. 2002, Ito et al. 2003b). Oak trees 20-50 cm [~8-20 in] diameter at breast height (dbh) and 20-30 m [~66-98 ft] tall wilt within 2-3 months after attack by the beetle carrying the fungus (Kubono and Ito 2002). Areas of mortality from Japanese oak disease (Ito et al. 2003b) appear to be expanding (Ito et al. 1998, Kamata et al. 2002, M. Yamato, pers. comm.). The pathogen, which has only recently been described (Kubono and Ito 2002), does not occur in the United States at this time and is of concern. The fungus is also a concern in Europe and appears on the European and Mediterranean Plant Protection Organization (EPPO) Alert List (EPPO 2005). The host status of oak species in the United States is not known. Nevertheless, given the widespread distribution of oak in this country, especially in the East (Smith et al. 2004), this pathogen poses a serious threat.

At this time, the only known hosts of *R. quercivora* are two species of *Quercus* that occur in Japan. One of these species, *Quercus mongolica*, occurs infrequently in ornamental plantings in the United States (USDA 2004), so environmental impacts from insect-pathogen attacks on this species would be minimal. If other *Quercus* spp. in the United States are susceptible to attack by *P. quercivorus* and infection by *R. quercivora*, the resulting environmental impacts could be significant.

Symptoms

In the early stages of disease, an *R. quercivora*-infected oak will have curled or withered leaves. Within a few weeks or less, the leaves become discolored and

begin to die. By the end of the season or the beginning of the next season, the tree is dead. Currently, *R. quercivora* is only known to be pathogenic on oaks in the white oak group. There are several diseases and abiotic factors that can cause curled or discolored leave. See 'Survey and Detection' for a more detailed description of these diseases.

Cross sections of trees infected with *R. quercivora* show galleries of *P. quercivorus* and discoloration that extends beyond the galleries into the surrounding sapwood (Kuroda 1998, Kobayashi et al. 2001). In the United States, oak wilt, caused by the well-established fungus *Ceratocystis fagacearum*, is vectored by beetles that do not make extensive galleries in the wood. The pattern of discoloration caused by *C. fagacearum* is different and begins as brown streaks that longitudinally follow the vessels in the outer sapwood (Sinclair et al. 1987, Juzwik et al. 2004). In both diseases, the discoloration in the sapwood becomes darker with time as tyloses plug the vessels.

Known Hosts

Raffaelea quercivora is only known to infect two species within the genus *Quercus*. Only one of these species, *Q. mongolica* var. *grosseserrata*, occurs in the United States and only in ornamental settings. Although other oak species are widespread in the United States, their host status is not known.

Raffaelea quercivora was identified from samples of discolored sapwood, inner bark and insect galleries of *Quercus serrata* and *Q. mongolica* var. *grosseserrata*. A single, small, host-range test suggests the fungus may be somewhat host-specific; seedlings of six species were inoculated with *R. quercivora*; three were deciduous - *Q. serrata, Q. mongolica var. grosseserrata*, *Q. acutissima*, and three were evergreen – *Q. acuta*, *Q. phyilliyraeoides*, and *Castanopsis cuspidata* var. *sieboldii* (Ito et al. 2003b). *Q. serrata* and *Q. mongolica var. grosseserrata* wilted within 10 days following inoculation, and *R. quercivora* was re-isolated from these trees. Five *Q. mongolica var. grosseserrata* and one *Q. serrata* died by the end of the experiment. No symptoms developed on seedlings of the other four species. Thus, not all *Quercus* spp. are equally susceptible to the pathogen, and the potential host status of oaks in the United States is highly uncertain.

Hosts	References

Quercus serrata (Japanese common name: ko-nara, bao li)
    (=*Q. glandulifera*)
    (=*Q. glandulifera* var. *brevipetiolata*)
    (=*Q. serrata* var. *brevipetiolata*)
    (=*Q. urticifolia* var. *brevipetiolata*)
	(Kubono and Ito 2002, Yamada et al. 2002, Ito et al. 2003b, CAB 2004)

Hosts	References
Q. mongolica var. grosseserrata (mongolian oak, meng gu li, mongori-nara) (=*Quercus crispula*) (=*Quercus grosseserrata*) (=*Quercus mongolica* subsp. *crispula*)	(Kubono and Ito 2002, Yamada et al. 2002, Ito et al. 2003b, CAB 2004, Kuroda et al. 2004)

Several *Quercus* spp. are hosts of other *Raffaelea* spp. A decline of Portuguese cork oak (*Quercus suber*) has been associated with outbreaks of *P. cylindrus*, which was found to carry several species of fungi into the trees, including *R. ambrosia* (Sousa et al. 1997). In most cases, however, ambrosia fungi have not been associated with symptoms or diseases of trees. Examples of non-pathogenic ambrosia fungi and their vectors found on *Quercus* spp. include: *R. ambrosiae* v. Arx & Hennebert, vectored by *Platypus cylindrus* in England (von Arx and Hennebert 1965); *R. ambrosiae*, vectored by *Platypus compositus* in Arkansas, United States (Batra 1967); *R. tritirachium* Batra, vectored by *Monarthium mali* in Pennsylvania, United States (Batra 1967); *R. montetyi,* vectored by *P. cylindrus* in France (Morelet 1998).

Known Distribution
The pathogen has only been reported from Europe and western Asia. *Raffaelea quercivora* has been identified from material collected in several prefectures in Honshu, Japan (Kubono and Ito 2002, Ichihara 2003, Kuroda 2005). *Raffaelea quercivora* may also occur in areas where mortality of Fagaceae, including *Quercus* spp., is associated with *P. quercivorus* attack. Identification of *R. quercivora* has not been reported from these areas, but the symptomatology and frequent presence of unidentified fungi in combination with high numbers of the vector suggest the presence of the pathogen. The distribution of oak mortality appears to be expanding in Ishikawa, Fukui and Shiga Prefectures (Fig. 10) (Kamata et al. 2002).

Location	References
Fukui Prefecture, Imajyomachi	(Kubono and Ito 2002)
Fukushima Prefecture	(Ichihara 2003)
Fukushima Prefecture,Takasato Village, Aizubange Cho	(Zhou undated)
Gifu Prefecture, Yanaitsu Cho[1]	(Zhou undated)
Hyogo Prefecture	(Ichihara 2003)
Ishikawa Prefecture[1]	(Ito et al. 1998)
Kii Peninsula	(Ichihara 2003)
Kyoto Prefecture	(Kuroda 2005)
Mie Prefecture	(Ichihara 2003)
Nara Prefecture	(Ichihara 2003)
Nigata Prefecture	(Ichihara 2003)

Location	References
Ryukyu Islands, Ishigaki Shima island[1]	(Hamaguchi and Goto 2003, CAB 2004)
Shiga Prefecture, Makino-cho (now Takashima City)	(Kubono and Ito 2002)
Shimane Prefecture	(Ichihara 2003)
Tottori Prefecture, Iwami-cho	(Kubono and Ito 2002)
Toyama Prefecture	(Ichihara 2003)
Yamagata Prefecture, Asahi-mura	(Kubono and Ito 2002)

1. These areas are identified as having oak mortality associated with *P. quercivorus* but isolation of *R. quercivora* from these areas was not attempted.
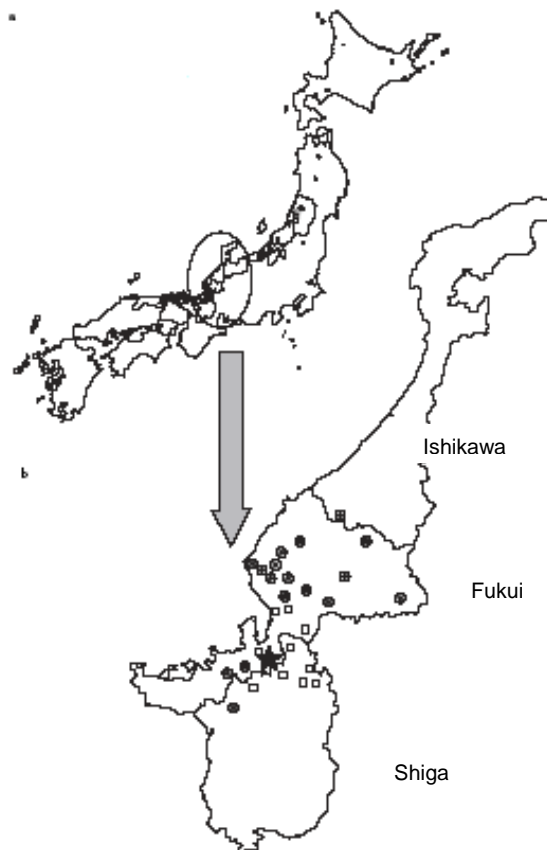

Fig. 10. Map showing occurrence of oak dieback caused by *Raffaelea* sp. 1 (now assumed to be *R. quercivora*) carried by *Platypus quercivorus* in three regions of Honshu, the central island of Japan (after Ito & Yamada, 1998). A detailed map of Ishikawa, Fukui, and Shiga Prefectures, showing localized spread of the disease from a focal point. Symbols denote periods when dieback was observed: ★ < 1980; □ 1980s; ◎ 1990s.
[Figure reproduced from Kamata (2002).]

Potential Distribution within the United States

Raffaelea quercivora occurs in central Japan, primarily on Honshu. The currently reported distribution of *R. quercivora* suggests that the pathogen may be most

closely associated with the temperate-broadleaf-and-mixed-forest biome, generally warm and humid during the growing season but with seasonal temperature and moisture fluctuations. Consequently, we estimate that 28% of the continental United States could provide a suitable climate for *R. quercivora*, extending from the northeastern-most states, south into parts of Louisiana and Texas, and west into Michigan, Wisconsin, and Minnesota.

A recent host map developed by USDA-APHIS-PPQ-CPHST (Fig. 11), illustrates the abundance of host material in the southeast as well as portions of the north east and western United States.
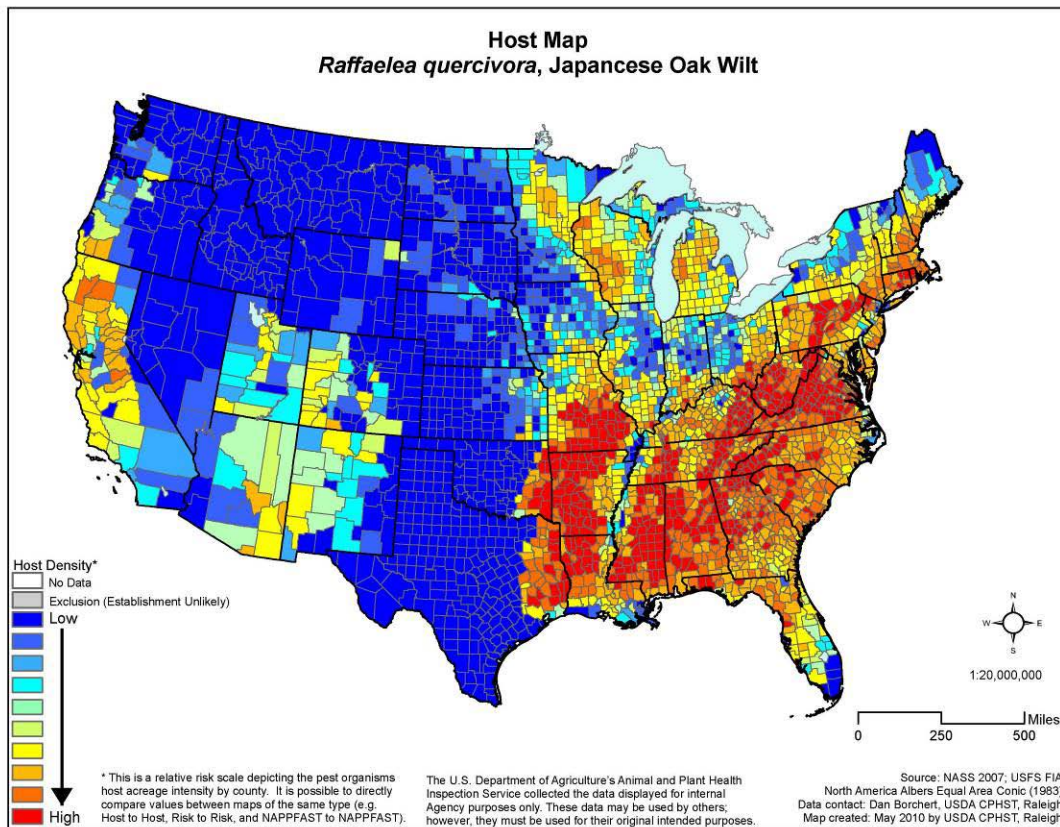
Fig. 11. Host map for *R. quercivora* within the continental United States. Values from low to high indicate increased host density. Map courtesy of USDA-APHIS-PPQ-CPHST. Check www.nappfast.org for the most recent map updates.

Survey
CAPS-Approved Method:
Visual survey is the approved survey method for *R. quercivora*. A survey for *R. quercivora* should target standing oak trees, oak logs and the vector, *Platypus quercivorus*.

A suspect tree or log should be cut in the field, and one or more cross sections should be examined for galleries of *P. quercivorus* and discoloration that extends beyond the galleries into the surrounding sapwood.

Literature-Based Methods:
A survey for *R. quercivora* should target standing oak trees, oak logs and the vector, *Platypus quercivorus*. Methods for monitoring *P. quercivorus* are described in the companion risk assessment by Davis et al. (2005). Because affected hosts and the insect vector may be associated with several species of fungi (Ito et al. 1998, Masuya et al. 1998, Kinuura 2002), identification of *R. quercivora* requires isolation and identification in a laboratory. Aerial surveys using photographs were used to define areas of oak mortality in Japan with some success (Kamata et al. 2001). Landsat imagery was too coarse to accurately identify these areas (Komura et al. 2003).

Only oak species are known hosts of *R. quercivora*, so they should be the focus of a survey. At this time it is unknown whether any oaks in the Unites States are susceptible. Tree crowns should be visually inspected for wilting leaves beginning in June through early September; leaves will appear curled or withered, then become discolored – to a reddish color in Japan (Saito et al. 2001, Kinuura 2002, Ito et al. 2003b, Kobayashi and Ueda 2003). Wilted trees may be dead by August or not until the following spring, and may appear in clusters. In Japan, centers of oak mortality are often found on the edge of a gap or stand (Esaki et al. 2004).

Wilted or dead oaks should be examined for evidence of attack by *P. quercivorus*: entrance holes, most dense within 1 m [~3 ft] of the ground (Hijii et al. 1991), and an accumulation of boring dust and frass at the base (Ichihara 2003). A suspect tree or log should be cut in the field, and one or more cross sections should be examined for galleries of *P. quercivorus* and discoloration that extends beyond the galleries into the surrounding sapwood (Kuroda 1998, Kobayashi et al. 2001). Sapwood discoloration in an infected tree was observed to a height of 4 m [13 ft] (Kuroda 2001). In Japan, sections of the trunk 20-30 cm [~8-12 inches] in diameter and 50 cm [20 inches] long were cut from felled trees or logs, and the ends were coated in the field with a silicone paste to prevent the wood from drying (Kinuura 2002). Sealed logs were taken to a laboratory for further analysis. Logs used as bait for *P. quercivorus* can also be used to detect *R. quercivora* (Kobayashi and Ueda 2003, Kobayashi et al. 2004).

Key Diagnostics
CAPS-Approved Method:
Confirmation of *R. quercivora* is by morphological identification. Identification will be based on the size and shape of conidiophores and conidia, and details of conidium production.

The colony is pale olive to brown and has a fragrance. The species is characterized by having small obovoid to pyriform sympodioconidia and slender, long conidiophores that taper to a point.

Raffaelea quercivora can be identified in the laboratory from the sapwood of an infected tree or log and from adult *P. quercivorus*. Surface sterilized pieces of discolored sapwood, insect galleries, adult beetles, and beetle mycangia can be plated on potato dextrose agar (PDA) with 100 ppm of streptomycin sulphate. Plates are incubated in the dark at 20-25°C (68-77 °F) for five days.

For easier isolation, it is recommended to aseptically remove slices of galleries and place them in a sterile moist chamber to encourage further growth of the 'ambrosia' fungus.

Literature-Based Methods:
Raffaelea quercivora can be identified in the laboratory from the sapwood of an infected tree or log and from adult *P. quercivorus*. Log samples were stored at 5°C [41°F], sawed into discs 2-4 cm [~1-2 inches] thick, cut into small blocks and split with a hatchet to expose the insect galleries (Kinuura 2002). For ambrosia fungi in general, Benjamin (2004) suggests preserving samples of thin slices or chips of galleries for later examination, either by drying or by mounting the samples on slides using a fixative mounting medium. Benjamin also suggests aseptically removing slices of galleries and putting them in a sterile moist chamber to encourage further growth of the ambrosia fungus for easier isolation.

A sterile scalpel is used to remove small pieces (e.g., cube 2-3 mm on a side) from the discolored sapwood and insect galleries (Kubono and Ito 2002). Samples are surface disinfected by washing with 80% ethanol and 0.1% solution of mercuric chloride and rinsed in two changes of sterilized water (Kubono and Ito 2002) or by rinsing each cube with 99% ethanol, heating over a flame, and repeating three times (Kinuura 2002). Once disinfected, the cubes are placed on plates of potato dextrose agar (PDA). Bacterial contamination may be inhibited with 100-ppm streptomycin sulphate solution applied to each plate (Kinuura 2002). Areas of discolored sapwood were the areas most consistently associated with isolation of the fungus (Kuroda 2001). It may also be possible to transfer pieces of the fungus directly from the gallery walls and pupal cradles to PDA (Kinuura 2002).

Isolation on PDA and incubation in the dark at 20-25°C [68-77°F] will produce colonies within 5 days (Kubono and Ito 2002). Identification will be based on the size and shape of conidiophores and conidia, and details of conidium production. Identification should be confirmed by scanning electron microscopy (Kubono and Ito 2002, Gebhardt and Oberwinkler 2005). A recent morphological comparison of several species of *Raffaelea* grown in culture highlighted that *R. quercivora* has more slender conidiophores and smaller conidia than other *Raffaelea* spp. (Kubono and Ito 2002).

To attempt isolation of the fungus from the insect vector, *P. quercivorus*, adult beetles should be surface disinfested by immersion in 80% ethanol for 30 seconds, rinsed in a dilute solution of sodium hypochlorite for 2 minutes, then

rinsed in sterile distilled water for 30 seconds (Kinuura 2002). Mycangia (Fig. 12) should be excised from female adults using a sharp scalpel or iris scissors and needles. Two or three mycangial sacs are placed on each plate of PDA. Proventriculi (terminal part of the foregut) should be removed from adults of both sexes and placed on PDA (Kinuura 2002). An alternative process is to wash the adults in two changes of sterilized water and place the entire insect directly on PDA (Kubono and Ito 2002). Bacterial contamination may be inhibited with 100-ppm streptomycin sulphate solution applied to each plate (Kinuura 2002). Plates should be incubated in the dark for 2 weeks at 25°C [77°F] or one month at 18°C [64°F] (Kinuura 2002, Kubono and Ito 2002). More than one fungus may be isolated from the beetle (Kinuura 1995). Timing and sex of insect collection can affect the isolation rate of *R. quercivora* (Kinuura 2002).
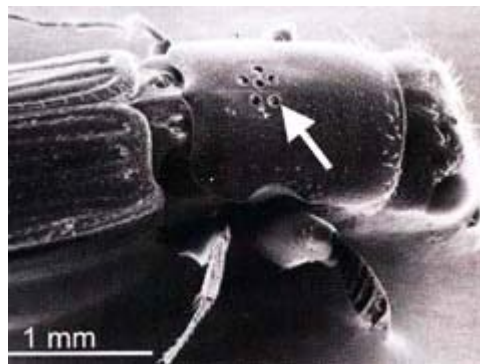

Fig. 12. Female *P. quercivorus* carries conidia in specialized cavities called mycangia (arrow).
[Photo from Kuroda (2005).]

Raffaelea. quercivora will grow rapidly (80 mm [~3 $\frac{1}{8}$ in] diameter in 5 days at 25°C [77°F]), and in 2 weeks will become pale olive to brown olive and have a fragrance (Kubono and Ito 2002). Isolates collected from many different oak trees showed no differences in culture morphology when grown on the same media (Kimura et al. 2005). Microscopic examination of conidiophores and conidia is required for identification, and scanning electron microscopy is better than light microscopy to observe conidiogenesis on fresh culture material (Gebhardt and Oberwinkler 2005). Confirmation should be made by an expert.

Identification of *R. quercivora* requires growth in pure culture. Within the near future, molecular methods may be available as another means of identification (Jones and Blackwell 1998, Kimura et al. 2005).

Easily Confused Pests
Two diseases that could be confused with the early foliar symptoms of Japanese oak disease are oak anthracnose and bacterial leaf scorch (BLS). Neither anthracnose nor BLS will cause rapid mortality like *R. quercivora*. Anthracnose is caused by the fungus *Apiognomonia errabunda* (imperfect state *Discula umbrinella*), and it occurs on many *Quercus* spp. in the United States (Tainter and Baker 1996, Gillman 1999). Spores, spread by rain and wind, infect leaves in spring, causing irregular blotches of dead tissue on leaves. Spots may coalesce

and entire leaves may shrivel, turn brown and die. Twigs may also be infected and die. Unlike *R. quercivora* infection, fruiting structures of the fungus may be seen (raised brown flecks) on the underside of lesions (Sinclair et al. 1987). Although unsightly and often recurrent, anthracnose is rarely a serious problem on established trees (Gillman 1999) and outbreaks usually diminish by mid-summer (Sinclair et al. 1987). BLS, caused by the bacterium *Xylella fastidiosa*, is another disease that occurs in oaks across the United States, and for which the foliar symptoms may resemble early symptoms of Japanese oak disease (Lashomb et al. 2003, Bentz et al. 2005). The bacterium is transmitted by insects (e.g., leafhoppers) and multiplies in the xylem, eventually blocking water conduction. Leaves show a marginal necrosis or browning, often with a distinct edge bordering the green tissue (Lashomb et al. 2003, Bentz et al. 2005). While symptoms of Japanese oak disease may be apparent early in the season (June) in Japan, BLS symptoms usually appear first in mid to late summer (Lashomb et al. 2003). Symptoms of BLS may occur on only one or a few branches in a season. Over time (years), branches and eventually whole trees may die. Leaves, twigs and branches can be tested for the bacterium by enzyme linked immuno-sorbent assay (ELISA) or polymerase chain reaction (PCR) (Bentz et al. 2005).

A third disease, oak wilt caused by the fungus *Ceratocystis fagacearum,* produces symptoms in red oaks over time that closely resemble those described for infection of *R. quercivora* (Juzwik et al. 2004). *Ceratocystis fagacearum* is vectored by sap and bark beetles and spreads through root grafts with other oaks. Hyphae grow in the xylem and sapwood, stimulate the formation of tyloses and block water conduction. Leaf discoloration and wilting begin in the upper crown within one or two months of infection, which usually occurs in late spring or early summer, and red oaks may be dead by the end of the season. Species in the white oak group are less susceptible, and may decline over many years before dying. Signs on the bark are different for oaks infected with *C. fagacearum* and oaks infected with *R. quercivora*. Red oaks infected with *C. fagacearum* may show small bark crack. If bark is removed, a gray mat of fungal mycelia may be uncovered. These fungal mats grow between the inner bark and the opposing wood, eventually creating enough pressure for the bark to crack (Juzwik et al. 2004). *R. quercivora*-infected trees have no such mats. Descriptions and images of other diseases and problems that produce symptoms similar to the oak wilt caused by *C. fagacearum* should also be reviewed to avoid confusion with disease caused by *R. quercivora* (Juzwik et al. 2004).

No other species of *Raffaelea* have been identified in association with *P. quercivorus*, but several *Raffaelea* spp. have been reported from the galleries and/or mycangia of other ambrosia beetles infecting *Quercus* spp. These fungi include *R. ambrosiae* in England and the United States (von Arx and Hennebert 1965), *R. montetyi* in France, (Morelet 1998), and *R. tritirachium* in the United States (Batra 1967). These species can be differentiated from *R. quercivora* based on the morphological characteristics outlined in the table below. In

general, *R. quercivora* has more slender conidiophores and smaller conidia than other *Raffaelea* spp., having conidia most similar in shape and size to *R. hennebertii* D.B. Scott et J.W. duTiot, vectored by *P. externedentatus* found in *Ficus sycomorus* in South Africa (Scott and Du Toit 1970). The conidiophores of *R. quercivora* are more slender than those of *R. hennebertii* (Kubono and Ito 2002), and the host and location where *R. hennebertii* occurs are very different.

Scanning electron microscopy, a method used to study *R. quercivora* (Kubono and Ito 2002), may be more useful than light microscopy to separate *Raffaelea* spp. based upon morphological characters (Gebhardt and Oberwinkler 2005). Molecular methods will soon be available to separate *R. quercivora* from other *Raffaelea* spp. (Jones and Blackwell 1998, Kimura et al. 2005).

Table 3. Comparison of morphological characters among *Raffaelea* spp.

Species	Sporodochia morphology	Conidiophore (µm)	Conidia (µm)	Conidia shape
Raffaelea quercivora[1]	Fascicles	16.5– 22.5 x 0.9–1.5	3.1–4.7 x 2.0–2.4	Obovoid–pyriform
R. ambrosiae[2]	Superficial	70–150 x 5–9	5–9 x 4–6	Obovoid–triangular
R. montetyi[3]	No description	No description	6.6–13 x 3–6.6	Obovoid–claviform
R. tritirachium[4]	Fascicles	6–15 x 3–5	5–10.4 x 1.5–2.5	Narrowly globose

1. Kubono and Ito (2002)
2. von Arx and Hennebert (1965)
3. Morelet (1998)
4. Batra (1967)

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