Ecological studies on *Trachysphaera lobata* (Ribaut, 1954) (Glomerida: Glomeridae) in the UK, with comments on the results of a taxonomic study

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Introduction

Trachysphaera Heller, 1858 is a genus of dwarf pill millipedes typified by a strongly ornamented and modified shape; they roll into tight balls that resemble tiny calcareous stones (Fig. 1) and it has also been suggested they resemble a small, white mass of fungi (D. Antič, pers. comm. regarding Facebook discussion). Dwarf pill millipedes are a challenge to study, their small size, and appearance like grains of sand, makes them difficult to find; in the experience of the authors often only a few specimens are found at any one time, but this may not be the situation in some caves (D. Antič, pers. comm.). Being tiny, their taxonomic features can be hard to see. Also, being pill millipedes they have telopods (instead of the gonopods found in most millipede groups) which are used to hold the females during mating rather than transfer sperm (Haacker, 1964), consisting of a chela and syncoxite. Their general morphological characters are also very variable, depending for example, upon developmental stage, amount of damage or proximity to moult (D. Antič, pers. comm.).



Figure 1: Habitus of *Trachysphaera lobata* from East Cliff. Photograph © J. Paul Richards.

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Trachysphaera is the third most diverse genus of the order Glomerida and is distributed mainly in the Euro-Mediterranean region, the Caucasus and reaching Germany, Poland and the UK in the north (Antić *et al.*, 2021).

Specimens of *Trachysphaera* were first found in the UK from East Cliff, Bembridge on the Isle of Wight, in 1984 (Jones & Keay, 1986). No males were present in this first collection and as the original description of this species uses male characters to separate *T. lobata* from *T. pyrenaica* (Ribaut, 1907) this made positive species identification difficult. However, they were identified as *T. lobata* using somatic characters from Demange (1981) and they were examined by J.-P. Mauriès at Paris Natural History Museum.

History of T. lobata in the UK

The report of the first finding at Bembridge also included that of a second site at the Duver in September 1984 (Jones & Keay, 1986). This has subsequently been recognised as an error, however the species was found in an area of scrub close to Bembridge Harbour a few hundred metres west of the East Cliff site, although this site has since been cleared and developed into a holiday park. The site at East Cliff was visited several times by Andy Keay and others and in 1986 *T. lobata* was found to occur in densities up to 5600 per m³ but only when selectively sampling small pockets of suitable substrate (Jones & Keay, 1986). In August 2004 three days of sampling yielded only two specimens and later that same year none were found during a further four days of fieldwork (Keay, 2004).

In 2010 *Trachysphaera* was reported from two locations in South Wales, one at Bynea, Llanelli in 2007 and one at Llanwrtyd in 2009 (Harper, 2010). The presence at the first location was confirmed in March 2011 by Ian Morgan (Wilbrandt *et al.*, 2015). In 2018 another population was discovered in caves in Prideaux Woods, Cornwall (Urquhart and Lee, 2019). Having mostly been collected in caves in France the habitat for this find was not unexpected.

Trachysphaera lobata was given the status RDBK (Red Data Book): Insufficiently Known, by Bratton (1991). In 2004 the British Myriapod and Isopod Group (BMIG) was given a grant by Natural England to improve understanding of this species and its ecology by undertaking field work on the Isle of Wight to establish its continued presence and the extent and density of the population. In 2011 the invertebrate conservation charity Hymettus made a successful bid to DEFRA to fund research on several Biodiversity Action Plan (BAP) priority species. One of the species was *T. lobata* and the British Myriapod and Isopod Group was asked to undertake the research. The work included the collection of specimens for the genetic studies reported on by Wilbrandt *et al.* (2015) in order to confirm the species of the populations in Wales and the Isle of Wight.

Methods

The site at Bembridge was visited on two separate occasions in 2005 and 2011. On 6-7 January 2005 a total of 22 man hours was spent in the field (Fig. 2). As specimens were quickly located, an estimate of the population density was carried out by counting the number present from a standardised volume (400cm³) of soil/leaf litter dug with a small trowel or with a bulb planter. Samples were passed through a sieve and the resulting fine material examined in the field. Samples were taken across the woodland from the sea shore to the base of the marl cliff and along the length of the woodland. Samples were not taken randomly and after a short time it was relatively easy to recognise the sandy, humus rich pockets of soil within which the species was found. The majority of the specimens were returned to the field after counting, but 54 were removed for morphological and life history studies, some were photographed and most are now preserved in the BMIG reference collection at Dinton Pastures. Two further days were spent searching woodland on the Isle of Wight that appeared, from maps, to be potential habitat for *Trachysphaera* based on similarity to the East Cliff woodland.



Figure 2: Searching for *Trachysphaera* at the East Cliff site.

On 22–24 February 2011 a further collecting trip was undertaken to East Cliff and non standardised hand searching was undertaken to collect 25 specimens for morphological and genetic studies (see below). The extent of the occupied area of habitat was assessed by searching for the millipede while moving east and west along the coast. In addition, one further woodland was searched as a potential *Trachysphaera* site.

The taxonomic and DNA part of this study has been published by Wilbrandt *et al.* (2015). Specimens from East Cliff collected in 2011 were sent with some from South Wales to the A. Koenig Research Museum in Bonn (ZFMK). A small number of specimens of *Trachysphaera* from other locations were also available for comparison. This included *T. lobata* from the Dordogne, France (collected by Desmond Kime); *T.* cf *pyrenaica* from Leitza collected as part of the BMIG field visit to the Basque area of Spain in 2009; *T.* cf. *pyrenaica* from Midi-Pyrenees, France (collected by Alex Schönhofer) and *T.* cf. *drescoi* from Aquitaine, France (collected by Alex Schönhofer).

In Bonn, scanning electron microscopy and multi-layer image photography were used to look at the male telopods, the anal shield, the anterior parts of the body including head, collum, thoracic shield and tergite 3 and the mid body tergites to compare ornamentation and colouration. DNA extraction was carried out on the same specimens.

Results

Population at East Cliff, Bembridge

The *T. lobata* site consisted of a narrow belt of semi-natural deciduous woodland dominated by Sycamore (*Acer pseudoplatanus*) with some Ash (*Fraxinus excelsior*) and Turkey Oak (*Quercus cerris*). The ground flora was dominated by mats of Ivy (*Hedera helix*), Nettle (*Urtica dioica*) and various grass species with some Bramble (*Rubus fruticosa* agg.), Dog's Mercury (*Mercuralis perennis*) and fern species.

In 2011 the woodland stretched for approximately 800m along the foot of an unconsolidated cliff approximately 7m high and facing roughly north-north-east. The maximum depth of woodland was approximately 50m and it ended in a wave eroded cliff between one and two metres high at the top of the beach. At either end of the woodland there were increasing amounts of human disturbance with gardens and buildings reaching down to the shore. The whole site is underlain by Bembridge limestone which outcrops approximately 30m offshore as low rock ledges. This is overlain by Bembridge Marls which form the platform on which the woodland grows. The cliff behind the woodland is a raised beach formed during the Ipswichian period. Sands and gravels eroded from the cliff cover the marls to varying depths and have created pockets of lighter soils within the heavy clay.

Within the area of woodland the population was not evenly distributed. The number of animals found within a 400cm³ sample of soil varied from 0 in most of the samples to 11 in one (Table 1).

Table 1: Numbers of *Trachysphaera lobata* collected from sampling locations at East Cliff in 2005.

Grid reference	40/647887	40/648887	40/649886	40/651885	Totals
No. of samples	19	40	14	8	81
No. of specimens	2	53	22	0	77

This patchiness of distribution appeared to be related to the nature of the substrate. *T. lobata* was only found in pockets of humus rich, sandy soil with or without coarser pebbles (Fig. 3). It was absent from any samples of clay soil or those taken from slumps at the base of the unconsolidated cliff where the soil was often sandy but lacked any organic material. No specimens were found in the litter layer and the millipede appeared to be most abundant at a depth of about 15cm, confirming observations by Jones and Keay (1986). Even where the substrate appeared suitable, many samples failed to produce any specimens.



Figure 3: Suitable substrate for *Trachysphaera.* 3 specimens are arrowed.

Based on the figures in Table 1 (77 individuals from 81 soil samples) this gives an estimated population density for the East Cliff woodland of 2377 individuals per cubic metre of soil. However, this figure significantly over-estimates the population size as it is based on samples selected from pockets of sandy soil considered the most likely to support the species. Using similarly selective techniques, Jones and Keay (1986) quoted a figure equivalent to 5600 individuals per cubic metre for the population density in 1984. This higher figure referred to a restricted area of the woodland in the centre of the range as determined by this study.

The extent of woodland occupied by *T. lobata* was found to be approximately 200m in length in 2005 and 120m longer to the east and 10m longer to the west in 2011. This increase of more than 50% does not necessarily mean an increase in population as the distribution within the woodland is uneven and was not quantified in any way in 2011. In addition, erosion continues on the shoreline and, while new cliff slumps are occurring on the inland side of the woodland it will take time for them to become suitable for the millipede. In addition, it is unknown if this new habitat is being generated at a greater or lesser rate than the shoreside erosion.

Although there appeared to be a healthy breeding population of *T. lobata* at Bembridge in 2005 there is evidence of a decline in population density over a 20-year period. In addition, the coastal strip is actively eroding and the woodland is slowly being washed into the sea. A large lump of concrete found isolated on the beach in 2005 was within the woodland in 1986 and it was estimated that the site has eroded by 5m in 15 years (0.33m/annum). Erosion rates around Bembridge are reported to be between 0.3 and 0.75m/annum generally with that at Tyne Hall, adjacent to the woodland where *Trachysphaera* was found, eroding at less than 0.15m/annum (Posford Duvivier, 1990). The woodland inhabited by *T. lobata* is less than 10 000 m² in area and is only approximately 50m in width (Fig. 4). Should the amount of erosion remain constant it will have disappeared in 150 years and suitable habitat for the millipede will have gone well before this.



Figure 4: Showing the narrow strip of woodland at East Cliff in which *Trachysphaera* has been found at East Cliff and the coastal erosion.

Other threats to the site include offshore dredging, extracting material for commercial use and the felling of trees to alleviate health and safety issues relating to the public footpath through the woodland. Loss of trees is likely to cause drying of the soil as well as decline in the amount of organic matter going into the soil each year.

Other sites on the Isle of Wight

The Bembridge Harbour site is now a holiday home development. Six standardised soil samples (400 cm³ each) were taken here but no *Trachysphaera* were found. Priory Woods, Hillway Woods, Centurion's Copse, Bembridge Down and Bembridge Lodge were the other woodlands visited. No specimens of *Trachysphaera* were found in any of these and only one, Centurion's Copse, appeared to have suitable conditions and might be worthy of further study.

Sex ratio and age structure of *T. lobata* collected in 2005

Of the 54 specimens collected, 40 were adult of which 11 were mature males. Previously no males had been found at Bembridge and it had been thought that the population might be parthenogenetic. However, the 3:1 female:male sex ratio in the samples from 2005 indicated otherwise. The mean diameter of mature males was 1.84 mm and of females with 11 tergites was 2.21 mm, as with many other species of millipede the males are slightly smaller than the females.

The remaining 14 specimens belonged to the two eldest immature stadia. The presence of juveniles suggests that the population is breeding. The small number of juveniles represented in the samples is probably a result of the difficulties in finding them as the smallest specimens collected were just 0.8 mm in diameter when enrolled and are thus very difficult to see in the field.

Male specimens were also found in the South Wales population. Examination of the male telopods from these and the East Cliff males showed slight differences from those in the type description.

Numbers and dimensions of T. lobata collected in 2005

The dimensions of those specimens collected are shown in Table 2. These are maximum diameter when enrolled.

Table 2: Dimensions of *T. lobata* specimens. Measurements are width of tergite 2 (thoracic shield) and whole diameter of rolled up animal in mm.

Number of tergites	Male	Female	Juvenile
			2 specimens
9			Tergite 2: 0.6
			Max diameter: 0.8
10			12 specimens
			Tergite 2: 0.6-1.0
			Max diameter: 0.9-1.1
	11 specimens	29 specimens	
	Tergite 2: 1.1-1.5	Tergite 2: 0.9-1.8	
	Max diameter: 1.5-2.2	Max diameter: 1.4-2.5	

Molecular analysis (results summarised from Wilbrandt et al., 2015)

The maximum likelihood tree supports the monophyly of *T. lobata* (97%) although the relationships between the three analysed populations are less clear (see Fig. 5).

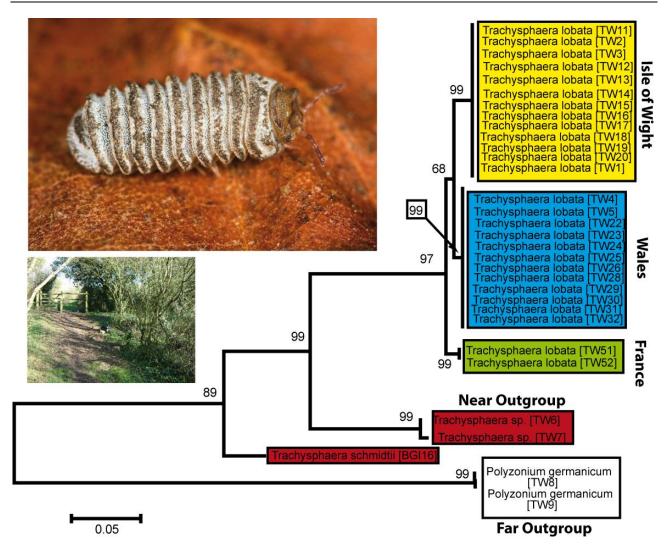


Figure 5: Maximum likelihood tree of 32 COI sequences of *Trachysphaera* and outgroup specimens (from Wilbrandt *et al.*, 2015)

Based on their mitochondrial DNA Wilbrandt *et al.* (2015) concluded that the UK *Trachysphaera* populations clearly belong to *T. lobata* and this was corroborated by the morphological data. The Isle of Wight and Welsh populations show unique haplotypes. The study was not able to trace the origin of the populations in the UK due to difficulties of getting more samples from France. However, the Isle of Wight and Welsh populations were found to have separate origins, although it is not known if this is the result of two separate anthropogenic introductions or two relic populations of a once more widespread distribution.

Morphological comparisons (summarised from Wilbrandt et al., 2015).

A total of 15 morphological characters commonly employed in the taxonomy of *Trachysphaera* were investigated for their taxonomic value, these included for example setae and grooves on the anal shield and the number of tubercles on the posterior margin of tergite 10.

Most of the somatic characters studied showed considerable variation, sometimes to a large degree, for example the number of toothed rows on the collum was variable on each side of the same individual. However, some characters were found that were constant between populations and species, such as the endotergum, or the underside of the tergites, a character that has proved helpful in the taxonomy of the giant pill millipede order Sphaerotheriida (VandenSpiegel *et al.*, 2002; Wesener, 2009).

The chela of the male telopods of individuals studied by Wilbrandt *et al.* (2015) showed huge variation within species, between species and even within the same individual, so they are unlikely to help with species identification, unlike in the Sphaerotheriida (Wesner, 2009).

Antić *et al.* (2021) concluded that the structure of the telopod syncoxite was the best character to determine species of *Trachysphaera*, in combination with some somatic traits, although this study did not include *T. lobata* it seems unlikely that this species would be significantly different.

Antič (pers. comm.) has also commented that the angle of view of the telopods is important and those of sub adult males are very different to those of mature males. There is also some difference of opinion over the clarity with which the telopods can be viewed using SEM, Wilbrandt, *et al.* (2015) concluded that this is helpful because of the ability to ensure the point of view is standard for all individuals examined. (See Fig. 6 which illustrates those parts of the male telopods mentioned here).

Therefore, the only characters that were consistent within a species but different between species were the number of rows of tubercles at the posterior margin of tergite 10 (*T. lobata* has two rows and *T. pyrenaica*, *T. cf. rousseti* and *T. cf. drescoi* have only a single row) and the presence of a protuberance on the anal shield in males (*T. lobata* lacks this and the other species have it). This, together with the telopod syncoxite should enable accurate species identification.

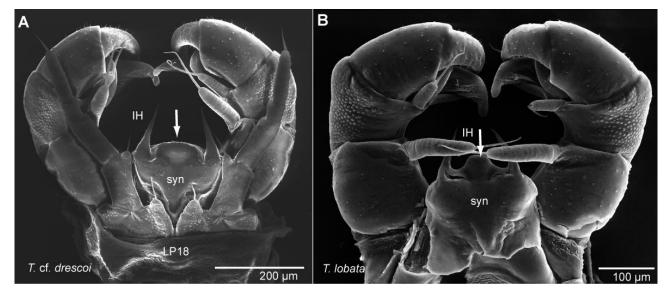


Figure 6: Scanning electron microscopy images of the telopod of *Trachysphaera* species, anterior view. A) *T.* cf. *crescoi* from Sare, France (ZFMK MYR 80), leg pair 18 with telopod; B) *T. lobata* from the Isle of Wight, U.K. (ZFMK MYR 898), telopod.

Arrows point to lobe of syncoxite (the only discernible difference in the telopods of both species). Abbreviations: IH = inner horns of syncoxite; LP18 = leg pair 18; syn = syncoxite of telopod.

Conclusions

The genetic studies (Wilbrandt *et al.*, 2015) have confirmed that this group of tiny millipedes are challenging to study and get good data from. However, they show that the British populations at Bembridge and Llanelli were referrable to *T. lobata*. The two population centres in the Isle of Wight and Wales are considered to be from separate origins although the reason for this is not known.

In 2005 the population at East Cliff, Bembridge included males, females and juveniles and was therefore presumed to be breeding successfully. While the population density had declined from that present in

1986 it was still likely to be a viable population, although restricted to small pockets of suitable substrate (areas with rich humus content as well as sandy elements) within a narrow strip of woodland.

Further searches for this species in Centurion's Copse would be worthwhile, as well as other places where the same rock type outcrops, for example on the west side of the Isle of Wight and in Dorset.

The current site at Bembridge is very vulnerable and is eroding fast. If the species could be found at a less vulnerable location then the future of *T. lobata* in the area would be more secure. Further findings of *T. lobata* at other sites in Wales and its recent discovery in Cornwall (Urquhart & Lee, 2019) are also helpful, lending support to the idea that it might be more widespread in the UK than previously thought and that exploration of caves/mines might be interesting. However, as species of *Trachysphaera* are so tiny in size with complex surface structures, new discoveries require careful checking to identify.

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