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European Journal of Protistology 81 (2021) 125789

European Journal of
PROTISTOLOGY

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The testate amoebae of New Zealand: A checklist, identification key and assessment of biogeographic patterns

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Received 17 July 2020; revised 25 February 2021; accepted in revised form 4 March 2021; Available online 30 March 2021

Abstract

New Zealand (NZ) is a well-known hotspot of biodiversity and endemism for macroscopic organisms, but its microbial diversity is comparatively poorly documented. We assembled all records on NZ testate amoebae published since the early 20th century and present a comprehensive taxonomic checklist for NZ. Testate amoebae are reported from six major habitat types across both the North and South Islands of NZ, but the sampling effort is ecologically and geographically biased in favour of wetlands and the South Island. As a result, 93% of all 128 morphotypes recorded in NZ occur in wetlands, 28% are restricted to the South Island, and diversity is greater at higher latitudes. Around 50% of morphotypes have a broad latitudinal distribution across the NZ mainland, whereas 15% have narrow latitudinal ranges. Future research should aim to broaden the geographical and ecological ranges. We predict that our list of NZ testate amoebae will expand substantially with future work, and that the latitudinal diversity gradient will be inverted. We also introduce an interactive, fully illustrated, online Lucid key for the rapid identification of NZ testate amoebae. As many morphospecies are cosmopolitan, this key provides a useful tool for testate amoebae identification in other parts of the world.

Keywords: Testate amoebae; New Zealand; Checklist; Identification key; Biogeography

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Introduction

Testate amoebae are a polyphyletic group belonging to three deeply divergent lineages: Amoebozoa (lobose testate amoebae) (Nikolaev et al., 2005) and the SAR clades (filose testate amoebae) Rhizaria (Bhattacharya et al., 1995; Dumack et al., 2016) and Stramenopiles (Gomaa et al., 2014). They represent one of the most diverse and abundant

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https://doi.org/10.1016/j.ejop.2021.125789
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groups of terrestrial protists. They are abundant in many terrestrial (soil, mosses, humus), semi-aquatic (peatland, swamps, marshes), freshwater (lakes, ponds, and rivers), and brackish environments (Charman et al., 1998; Gehrels et al., 2001; Mitchell et al., 2008). Their sensitivity to fine-scale environmental gradients (Balik, 1996; Jung, 1936; Mieczan, 2010; Mitchell et al., 2000; Tolonen et al., 1985) and minor alterations in environmental conditions (Whittle et al., 2019; Lousier, 1974) means that they are excellent bioindicators for assessing the ecological integrity of peatlands (Beaulne et al., 2018; McKeown et al., 2019), to assess the effect of restoration (Creevy et al., 2018; Swindles et al., 2016; Valentine et al., 2013), as well as other applications such as dating cadavers (Seppey et al., 2016; Szelecz et al., 2014), assessing the impact of pesticides on agricultural soils (Foissner, 1999; Heger et al., 2012), lake pollution (Reinhardt et al., 1998) and as model organisms in ecotoxicology (Amacker et al., 2018; Meyer et al., 2013). Testate amoebae are also a useful group of model organisms for the study of microbial biogeography (Smith et al., 2008).

Testate amoebae produce decay-resistant tests (shells) that allow reconstruction of past hydrological conditions from peatlands (Amesbury et al., 2016; Booth et al., 2006; Charman, 1997; Hendon et al., 2001; Swindles et al., 2019; Tolonen et al., 1985; Wilmshurst et al., 2002). They have also been proposed as proxies for reconstructing sea-level fluctuations (Gehrels et al., 2001) and past volcanic eruptions (Delaine et al., 2016). Traditional identification of testate amoebae relies on characteristics of the test, such as size, number of apertures, shape, and composition (Ogden and Hedley, 1980). Tests are composed of a proteinaceous matrix (amorphous, e.g. *Hyalosphenia*, or plate-like, e.g. *Arcella*), which can be reinforced with agglutinated extraneous material (referred to as xenosomes, e.g. *Difflugia*), self-secreted calcareous or siliceous plates (referred to as idiosomes, e.g. *Paraquadrula*, *Euglypha*) or siliceous plates recycled from consumed prey (e.g. *Nebela*). Some taxa can incorporate both xenosomes and idiosomes (e.g. *Netzelia*), or xenosomes and recycled siliceous scales (e.g. *Heleopera*) into their tests.

The study of testate amoebae from New Zealand (NZ) began in the early 20th century with samples collected as part of the 1907–9 British Antarctic Expedition (the Nimrod Expedition) led by Ernest Shackleton, which were sent by expedition biologist James Murray to Swiss protozoologist Eugène Penard (Penard, 1911). Two testate amoeba species, *Hyalosphenia cockayni* and *Nebela certesi* were described from these samples. Almost two decades later, Brehm (1928) reported a new species, *Nebela penardi*, from samples of *Sphagnum* and algae collected from a small wetland near Arthur's Pass in the South Island. This species resembles *Apodera vas* but bears a distinctive keel as in genus *Gibbocarina*. Although no measurements were provided in the description, the line drawing is unambiguous and confirms that this is a new taxon. As this description was hidden at the end of a study describing micro-crustaceans it was overlooked by subsequent researchers. The taxonomy of this species will be clarified elsewhere. Two decades later, Hoogenraad and De Groot (1948) and Van Oye (1956), reported and described new testate amoebae from New Zealand wetlands (bogs, including *Sphagnum* mosses, swamps, and small ponds). Deevey (1955) report three genera of testate amoebae in a palaeolimnological investigation of an upper swamp deposit from Pyramid Valley in Canterbury, South Island. Testate amoebae were then reported from two streams in the Taupo geothermal region by Winterbourn and Brown (1967) and from agricultural soils, grasslands, tussocks, and ephemeral ponds by Stout (1984). Since the turn of the 21st century, several studies have focused on the taxonomy, ecology and palaeohydrological applications of testate amoebae (Bamforth, 2015; Charman, 1997; McGlone and Wilmshurst, 1999; McKeown et al., 2019; Wilmshurst et al., 2002, 2003). These studies increased the number of species recorded in New Zealand considerably.

The development of a testate amoeba-based training set from peatlands spanning the North and South Islands provided a tool for reconstructing past water table depth and soil moisture (Charman, 1997), which was then used to reconstruct past hydrological conditions from two southern peatlands. (McGlone and Wilmshurst, 1999; Wilmshurst et al., 2002, 2003). Hazell (2004) conducted a palaeoecological study of two ombrotrophic peat bogs in the Waikato region of the North Island, but poor preservation of tests prevented adequate past moisture reconstruction. Recently, the smaller (<45 μm) testate amoebae were shown to be useful ecological indicators in variously degraded peatlands (McKeown et al., 2019).

Microbial community diversity has been shown to be shaped across space and time by a combination of environmental selection, diversification, stochastic drifts, and dispersal limitation (Lear et al., 2017; Hanson et al., 2012). In contrast to earlier views that all microbes had a cosmopolitan distribution, a selected number of larger 'flagship' protist taxa, including some conspicuous and relatively large testate amoebae (>100 μm) were shown to have restricted geographical distribution (Foissner, 2006; Smith et al., 2008). *Apodera vas* is the most frequently cited example, being almost entirely restricted to former Gondwana and subantarctic islands (Smith and Wilkinson, 2007). Smaller testate amoebae appear to be cosmopolitan in distribution based on morphological identification. However, although a dispersal modelling study suggested that only the smallest taxa (<40 μm) are likely to travel across continents, inter-hemispheric transport of these taxa is unlikely (Wilkinson et al., 2012). Furthermore, the smallest taxa may hide pseudo-cryptic diversity as shown for the *Euglypha rotunda* complex in a study combining SEM imaging and DNA barcoding (Wylezich et al., 2002) and this diversity may reveal contrasted geographical distribution or ecological preferences, as shown for the group of *Nebela tincta* in peatlands (Singer et al., 2018). A major challenge in microbial biogeography is to ascertain the absence of a taxon from a given area. And here the case of “Gondwanan” testate amoebae (genera *Apodera, Alocodera* and *Certesella*) is especially interesting as these large taxa have very characteristic morphologies (Heger et al., 2011). Furthermore, these taxa are mostly found in *Sphagnum*-dominated peatlands and forest litter, which are abundant habitats in temperate regions of the Northern Hemisphere that have been intensively studied for testate amoebae; it is therefore impossible to miss them even for a beginner.

New Zealand is a hotspot of endemism (Daugherty et al., 1993) with a distinctive modern biota that includes both vicariant Gondwanan elements (Cooper and Millener, 1993), as well as more recently dispersed fauna and flora (McGlone, 2005). The isolated landmass of New Zealand is 1635 km east of mainland Australia and stretches over 1600 km from latitude 34° S to 47° S. The geological history (emergent part of the continent Zealandia) and variety of biological processes could potentially explain the origin of its unique biota (Cooper and Millener, 1993). However, an assessment of endemism is missing for microorganisms.

In order to help fill this knowledge gap and guide future testate amoebae research, we provide the first checklist for the testate amoebae of New Zealand and include updated nomenclature following recent phylogenetic and morphological studies. We also provide the first overall assessment of diversity and geographic patterns for New Zealand testate amoebae using a qualitative and quantitative approach that explores species richness, habitat preferences and latitudinal distributions.

To facilitate further research and to ensure consistency of taxonomic nomenclature in future studies, we also provide a reliable and easy to use identification tool for New Zealand testate amoebae. This freely available, online, interactive identification key allows for regular taxonomic updates, and provides a valuable resource to the scientific community and training aid. Designed as a Lucid key (Norton et al., 2012), the tool is interactive and fully illustrated, and require significantly less expertise to use than traditional dichotomous keys (for example, the most frequently cited reference guide by Charman et al. (2000)). This key uses diagnostic features of tests (dimensions, structure, length, width, shape, colour, aperture shape and number) to discriminate and identify taxa to genus and species level. As there are few fully illustrated identification keys freely available online (apart from https://www.arcella.nl), our New Zealand-based testate amoebae Lucid Key fills a niche. Because many New Zealand taxa also occur elsewhere in the Southern Hemisphere or are cosmopolitan, our key will also have more widespread application and can easily be updated and augmented as new data are generated.

Materials and methods

Geographical setting

The New Zealand archipelago lies in the South Pacific Ocean, stretching over 1600 km from latitude 34° S to 47° S and is isolated from other significant landmasses by >1500 km of ocean. Although appearing as an archipelago, New Zealand is the emergent part of the continent Zealandia; a section of continental crust that split and rotated from the eastern margin of Gondwana ~80 million years ago (Neall and Trewick, 2008). The North and South islands form the largest landmasses of NZ and the relatively young axial ranges (~10 million years) form a chain of tall mountains which run the length of the South Island up to the Volcanic Plateau in the central North Island.

Database construction

We compiled published and unpublished information documenting testate amoebae from all habitat types across New Zealand. The construction of the database was carried out in two steps: (1) a literature review was undertaken to collate all published material from research related to testate amoebae of New Zealand; (2) additional surface samples (157 samples from 33 sites) from wetlands (specifically bogs) in the North and South islands were collected and analysed for testate amoebae to expand the geographic coverage of the database (Fig. 1). The sites included a range of near-pristine to degraded bogs (the latter evident from associated artificial drains, area loss and fragmentation). This field work was carried out in four separate expeditions with three taking place in the summer months between 2015 and 2019, and one sampling excursion in the winter of 2018. From each site we collected surface vegetation and peat samples. We used a knife to cut into the surface carpet of vegetation and removed a 20-cm deep intact monolith. This section was inspected, and a sample was taken from the surface to capture the living section of the carpet and from the top 0.5 cm of surface peat. At each site, we extracted a sample from one of three micro-topographical features (i.e., hummock, hollow and lawn), where all features were captured. Between three to six samples were extracted from each site.

Laboratory methods

We followed established protocols for extracting testate amoebae from surface peat samples (Charman, 1997; Hendon and Charman, 1997; Mitchell et al., 1999; Woodland et al., 1998). Approximately 30 g of the surface vegetation and near surface peat was collected, placed in a sealed container with tap water and shaken for at least two minutes with occasional stirring. The samples were sieved

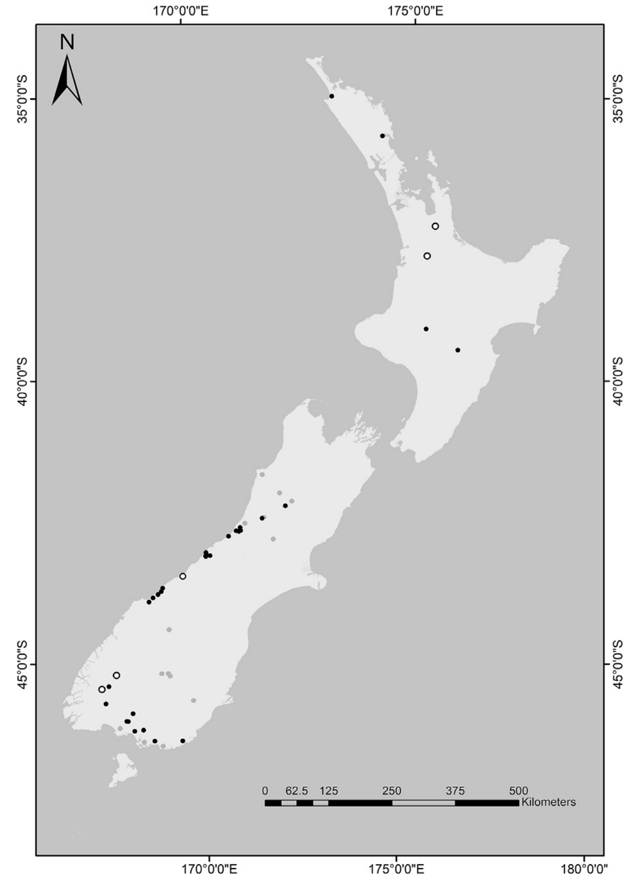

Fig. 1. Map of New Zealand including the North and South Islands. The grey dots represent peatlands that were sampled for testate amoebae community composition in the published work by Charman (1997), McGlone and Wilmshurst (1999), Wilmshurst et al. (2003, 2002). The black dots represent the additional study sites that were sampled as part of this study. The white dots represent sites that were sampled previously by the aforementioned studies and that were sampled again in this study.

through a 250 µm mesh and then through a 150 µm mesh to remove larger fragments, and then back-sieved over a 15 µm mesh to remove fine particles. Thus, the 15–150 µm fraction was placed in a 50 mL centrifuge tube and centrifuged for three minutes at 3000 rpm to concentrate the tests. The concentrate was transferred to a 2 mL vial and preserved in 75% ethanol.

Amoebae tests were identified at 200–600 × magnification using a Nikon Eclipse 80i. A minimum of 150 individuals (living and dead) were counted from each sample. Light microscopy photographs of all morphotypes were taken in wide-field of view and morphometric features were measured using NIS-Elements D 5.01.00 64-bit. Specimens were identified to the finest possible taxonomic resolution using several keys and monographs including Charman et al. (2000), Corbet (1973), Deflandre (1936), Hoogenraad and De Groot (1948), Leidy (1879), Mazei and Tsyganov (2006), Ogden and Hedley (1980), Penard (1902), Van Oye (1956). For scanning electron microscopy (SEM), 1-mL of sample was placed in a plankton counting tray and testate amoebae were handpicked on an inverted microscope (Olympus IX83). Specimens were placed on cover slips that were mounted on stubs and finally ethanol and air-dried. The stubs were coated in gold and photographed with a Hitachi TM3030plus Scanning Electron Microscope operating at 5–15 kV.

We followed the graphical and statistical methods used by Fernández et al. (2015) to assess the patterns of diversity and distribution of New Zealand testate amoebae. As data were compiled from sources that used various methods and taxonomic approaches, taxonomic revision of some genera and species, based on recent molecular and phylogenetic studies, was necessary (Chatelain et al., 2013; Gomaa et al., 2017, 2013, 2012; Heger et al., 2013; Kosakyan et al., 2016a, 2016b, 2013, 2012; Lahr et al., 2013, 2012; Lara et al., 2008, 2007; Mazei and Warren, 2012). We also noted the habitat(s) where each taxon had been recorded along with their geographical distribution(s). Where possible, we incorporated recent taxonomic revisions into the new checklist of valid testate amoeba taxa recorded in New Zealand to ensure nomenclature harmonisation between new and older literature (Supplementary Table 1) (Adl et al., 2019; Duckert et al., 2018; Gomaa et al., 2013; Kosakyan et al., 2016a, 2013, 2012, 2016b; Lahr et al., 2013, 2012).

Testate amoebae richness, diversity and biogeographic analysis

The information in the database (provided as online supplementary material to this paper) was used to calculate the total number of taxa recorded across New Zealand, including genera, morphospecies, infraspecific taxa and unidentified species. As we do not know how test morphology and genetic diversity are related for most taxa, we use the term morphospecies instead of species. We also use the term morphotype to informally describe a sub-set within a species/genus. We then explored diversity patterns across both the North and South Islands, and the number of published papers describing and recording taxa over the last 100 years. Calculations were performed on Amoebozoan and SAR testate amoebae separately and together. Species accumulation curves were plotted to explore the evolution of knowledge about the New Zealand testate amoebae over time. Species accumulation curves with 95% confidence intervals (based on a bootstrap method with 20,000 replications) were calculated for the peatland habitat to determine the completeness of the inventory, i.e. if we are close to reaching known testate amoebae diversity from New Zealand peatlands. This could not be carried out for the other habitats as there was insufficient information in the corresponding literature. This analysis was calculated using R version 3.6.3 (R Core Team, 2013, 2018) using vegan package 2.4.5 (Oksanen et al., 2013) and BiodiversityR package 2.12–3 (Kindt and Kindt, 2015).

To investigate testate amoebae habitat preference, we collated information on habitats from the literature and from our own investigation. We classified each habitat as terrestrial, aquatic and semi-aquatic. This also highlights the habitats that are under-represented in the database and identifies areas that would benefit from future work. The total number of morphotypes were reported for each habitat, along with the Amoebozoa and SAR groups. To determine if testate amoeba morphotypes presented a latitudinal pattern in their distribution, the presence and absence of each taxon were recorded in latitudinal bands of 1° across New Zealand (46° S to 35° S = 11 latitudinal bands). These data were used to construct a binary matrix spanning the latitudinal bands and 115 taxa. Not all morphotypes in the database could be mapped due to a limitation in information in the literature. A map of New Zealand illustrating species distribution was created using presence and absence with a resolution of 1° latitude using ArcGIS 10.6.1. We assumed that geographical ranges were continuous between latitudinal bands, as proposed in Fernández et al. (2015).

Lucid key construction

To improve the identification process of testate amoebae we developed an on-line interactive Lucid Key based on the new species checklist. The key was constructed using the program Lucid 3.6 Builder (Norton et al., 2012). Information on test morphology was taken from the literature, original monographs and our own observations and measurements. The information for each taxon was then entered into the Lucid 3.6 Builder through a scoring system, which allows diagnostic features of tests to be selected to narrow down possible groups. This differs from traditional taxonomic keys that use dichotomous decisions, which requires all features to be assessed and could prevent optimal identification if one is missing due to degradation of the test. Thus, the Lucid Key may be preferable for palaeoecological work as degraded tests have a better chance of being identified.

In the Lucid Key, the user selects a specific character and all the taxa that have that character are retained and those that do not are discarded. A limited sub-set of taxa are presented each time a character is selected. For example, the *position of aperture* feature has the three states: *terminal*, *sub-terminal / off-central*, *central*. When *terminal* is selected it retains 89 morphotypes with this feature and removes 39 morphotypes that do not have a terminal aperture. The process is repeated until the user is left with a small number of species, which simplifies the identification process. We have built the key to account for common misconceptions or misinterpretations when identifying a character that can be easily confused. For example, the feature *test shape* has seven different states, and if the user selects the *ovoid* state but the test being identified can be commonly misinterpreted as *cylindrical/oblong* then the species of interest will not be discarded if the latter state is selected. This avoids incorrectly eliminating the corresponding taxa during the identification process. To further facilitate identification by non-experts, an image highlighting the characteristic of interest was provided (i.e., test shape). Illustrations are digitised images from monographs, original images taken as part of this study, or images from the online resource www.arcella.nl/.

Results

Testate amoeba diversity in New Zealand

We have identified 30 new morphotypes that were not previously recorded in New Zealand (comprising 19 genera, 29 morphospecies, two of which are infraspecific taxa) (Supplementary Tables 1-2). One morphotype within the genus *Certesella* displayed a notably different morphology to other morphospecies in this genus and is likely a new species but phylogenetic work is necessary to confirm this. We refer to this morphotype as “*Certesella* sp. a” and do not formally describe it in this study. It was difficult to determine if any taxa from previous studies were misidentified as we did not have access to re-examine the original data or illustrations. Moreover, the samples (testate amoebae transferred to slides) that we did have access to re-examine had become too degraded. After ensuring consistent nomenclature, the total number of testate amoebae recorded in New Zealand was 128 taxa, of which 107 could be classified to morphospecies level, belonging to four phyla, four classes, four orders, 18 families, 37 genera.

The number of taxa recorded in New Zealand has steadily increased over time without any sign of plateauing (Fig. 2). The assessment of the inventory for peatland testate amoeba species richness indicates that the current sampling effort (i.e., number of samples) is insufficient to detect the true diversity although a plateau starts to be visible (Fig. 3). Testate amoebae began to be recorded in 1911, with renewed interest in the mid-20th century (Fig. 2a–c). As to be expected, there is a notable increase in the number of taxa recorded with each study in both the Amoebozoa (Fig. 2b) and SAR groups (Fig. 2c). Renewed interest in these organisms in the late 1990s resulted in a 30% increase in recorded taxa since 1983. This pattern was explored for the Amoebozoa and SAR groups over this time-period, with an increase in 26% (20 taxa) and 17% (4 taxa), respectively (Charman, 1997). Between 1999 and 2004, four studies with a palaeoecological application revealed yet more taxa (14% in all taxa, 12% in Amoebozoa and 4% in SAR) (Hazell, 2004; McGlone and Wilmshurst, 1999; Wilmshurst et al., 2002, 2003).

A more detailed comparison was carried out between our new data presented here, and data from Charman (1997), McGlone and Wilmshurst (1999), Wilmshurst et al. (2002), Wilmshurst et al. (2003). All samples were

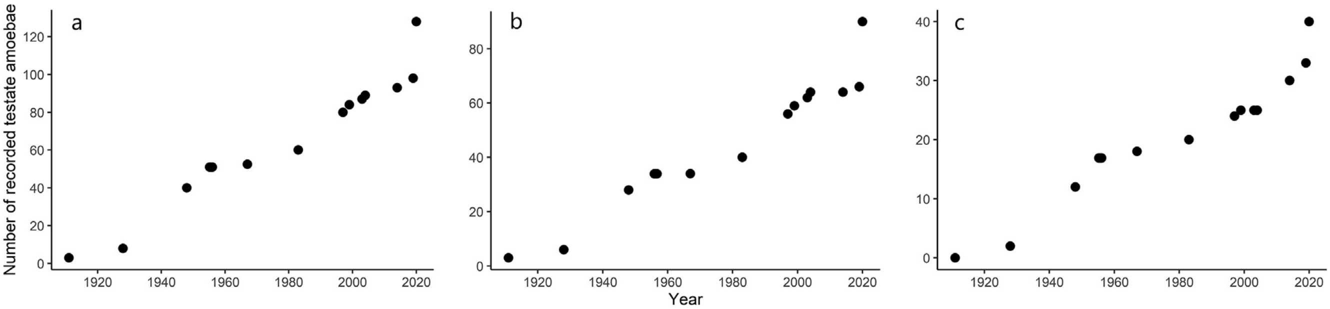

Fig. 2. Additive number of testate amoeba taxa recorded in New Zealand over time for (a) all taxa, (b) amoebozoan taxa, (c) SAR taxa.

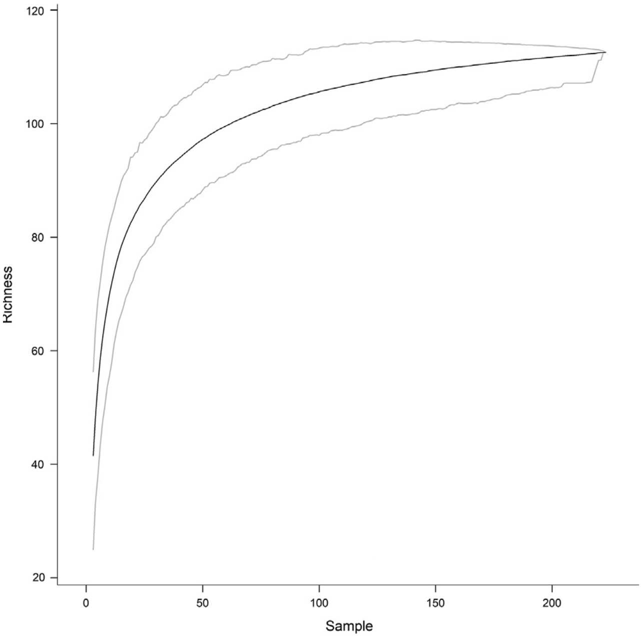

Fig. 3. Species accumulation curve with 95% confidence intervals (based on a bootstrap method with 20,000 replications) for peatland samples only.

collected from wetlands (specifically bogs) across both the North and South Islands of New Zealand. The comparison was carried out on modern samples only (i.e., surface peat samples) to examine if recent advances in taxonomy have altered the percentage of taxa found in the different genera in the Amoebozoa and SAR groups across wetlands (Fig. 4a and b). The percentage of Amoebozoa and SAR taxa was similar in our new data and the previously collected data; the total taxa in the Amoebozoa (and SAR supergroup) was 56% (SAR was 44%) in our new dataset (Fig. 4b) compared to 55% (SAR was 45%) in the previously collected data (Fig. 4a). However, our new data shows that there is a greater relative number of smaller (<45 µm) testate amoebae species and morphotypes recorded than previously known. To test if this was not an artifact of different methodologies, at our initial stages in developing a New Zealand specific testate amoeba sieving protocol, we analysed the sample captured between the 250 µm and 150 µm. We did this randomly throughout our analysis to ensure that we were not missing any taxa. We could not find any larger testate amoebae at this level, indicating that there is a lack of testate amoeba taxa in New Zealand at this larger fraction from wetlands. It should be noted that a 150 square mesh theoretically allows particles with a width of $150\ \mu m \times \sqrt{2} = 212.13\ \mu m$ to pass. Very few soil and moss-dwelling testate amoeba species are indeed wider than this.

Ecological and distribution patterns of testate amoebae richness and diversity

Testate amoebae have been recorded from only six main habitats in New Zealand (Table 1). Of these, two are classified as terrestrial (temperate forest litter and soil, grasslands), two semi-aquatic (wetlands – bogs and some swamp samples, *Sphagnum* moss from wetlands), and two aquatic (geothermal stream, ephemeral pond). The greatest number of recorded testate amoebae was in wetlands (119 morphospecies/morphotypes, 93% of the recorded total for New Zealand); however, it must be noted that most research on these organisms has been carried out on this habitat. In *Sphagnum* samples, 34 morphospecies/morphotypes were recorded making up 27% of the total recorded morphotypes for New Zealand; in forest soil and litter 39 morphospecies/morphotypes were recorded (30% of the NZ total); in grasslands 13 morphospecies/morphotypes were recorded (10% of the NZ total); in ephemeral ponds 9 morphospecies/morphotypes were recorded (7% of the NZ total); in geothermal streams 3 morphospecies/morphotypes were recorded (2% of the NZ total) (Table 1 and Fig. 5a; Supplementary Table 2).

The largest number of Amoebozoan taxa were found in wetlands (86 morphospecies/morphotypes), followed by *Sphagnum* moss (25), temperate forest litter and soil (22), grasslands (4), ephemeral ponds (4), and geothermal streams (2) (Fig. 5a). A lower number of SAR taxa were observed compared to Amoebozoan testate amoebae in all habitats (wetland = 33, *Sphagnum* = 6, temperate forest litter and soil = 17, geothermal stream = 2) apart from grasslands (9) and ephemeral pond habitats (5) (Fig. 5a).

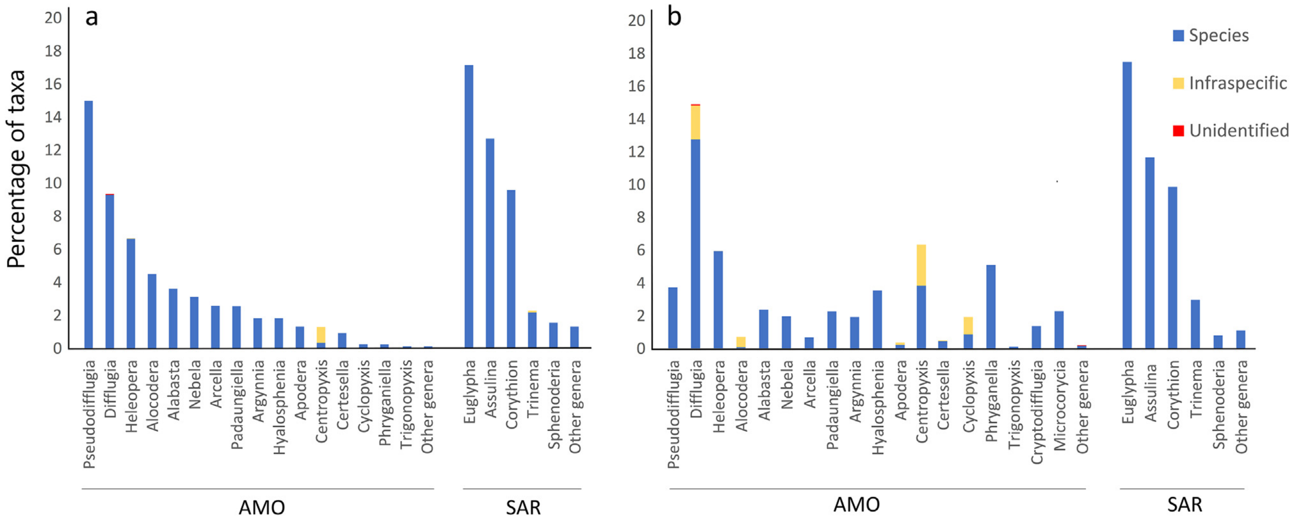

Fig. 4. Percentage of taxa in groups based on genera included in each amoebozoan genus and SAR genus for (a) peatland work carried out by Charman (1997), McGlone and Wilmshurst (1999), Wilmshurst et al. (2003, 2002), and (b) the additional peatlands presented for the first time in this study.

Table 1. Habitats explored to study the New Zealand testate amoebae, including amoebozoan testate amoebae (AMO), SAR testate amoebae, and the total number (TA) of morphospecies/morphotypes and percentage that have been recorded in each habitat.

Type	Habitat	TA (no.)	TA (%)	AMO (no.)	AMO (%)	SAR (no.)	SAR (%)
Terrestrial	Temperate forest litter and soil	39	30	22	17	17	13
	Grassland	13	10	4	3	9	7
Aquatic	Thermal stream	3	2	2	1.5	1	< 1
	Ephemeral pond	9	7	4	3	5	4
Semi-aquatic	Wetland (mostly bog but some swamp samples)	119	93	86	67	33	26
	Sphagnum moss	34	27	25	20	6	5

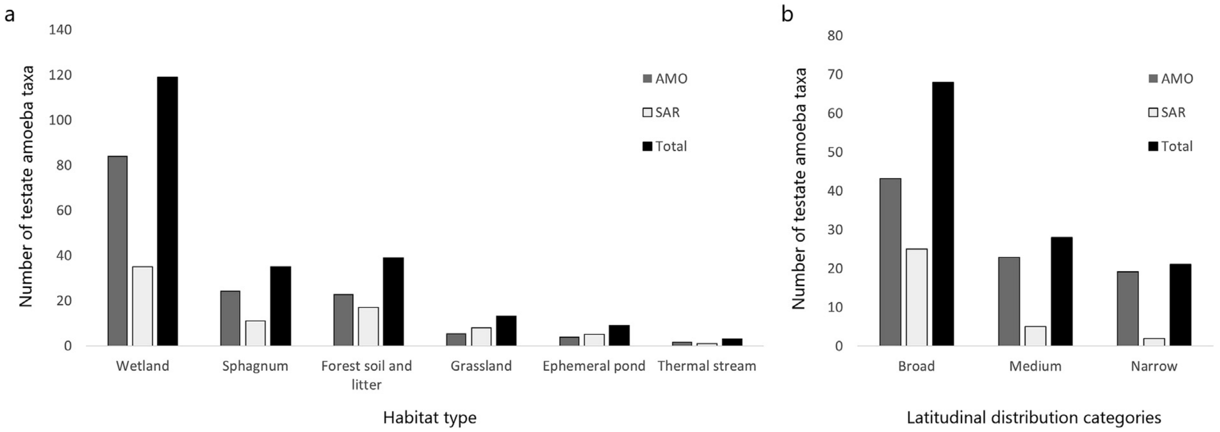

Fig. 5. Testate amoebae morphotypes included in amoebozoan and SAR categories along with total values for (a) different habitat types, and (b) latitudinal categories where large = 11–9 latitudinal bands, medium = 4–5 latitudinal bands, narrow = 1–2 latitudinal bands.

The analysis of latitudinal patterns of testate amoebae showed that 50% of morphospecies/morphotypes are widespread across New Zealand (between seven to 11 latitudinal bands, which we refer to as broad distribution) (Fig. 5b and Fig. 6). Around 20% of morphospecies/morphotypes were found to have a medium distribution (4–5 latitudinal bands), only 15% were found to have a more restricted latitudinal range (1–2 latitudinal bands, which we refer to as narrow distribution) (Figs. 5b and 6). Around 29% of morphospecies/morphotypes were recorded only in the South Island.

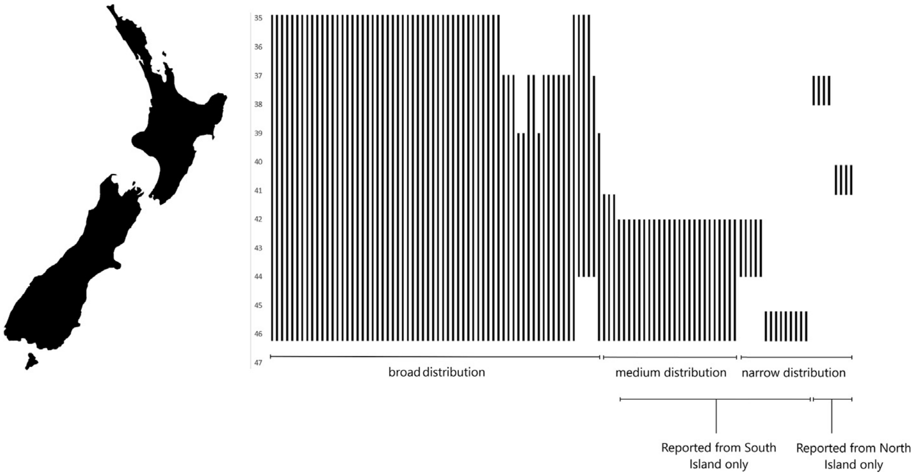

Fig. 6. Latitudinal distribution for 115 testate amoebae taxa in New Zealand. This includes all known patterns for species in peatlands, grasslands, and temperate forest litter and soils. Each vertical band represents the distribution of a single species. See Supplementary Table 2 for species details.

However, this may be due to a sampling bias, where the South Island has been more extensively sampled than the North Island. The majority of morphospecies/morphotypes considered Gondwanan or endemic (*Apodera vas, Alocodera cockayni, Nebela penardi, Certesella* spp.) and small taxa (<45 µm) have a large latitudinal range.

We identified a number of taxa with possible Gondwanan origins including *Apodera vas, Nebela penardi, Alocodera cockayni,* and *Certesella* spp. (illustrated in Fig. 7). Some of these taxa have only previously been described in poor quality images and/or hand drawings. Following detailed analysis of the Gondwana-specific taxa, we also note two different morphotypes of *Apodera vas* for New Zealand; a narrow morphotype (*A. vas* – narrow type) and one that appears to be broader (*A. vas* – broad type) as described by Penard (1911) (Fig. 7a.1-a.2 and Supplementary Table 1). All morphotypes have a broad geographic distribution across New Zealand wetlands and *A. vas* has been commonly found in temperate forest soils and litters. The morphological diversity within the genus *Apodera* has also been noted in Chile (Fernández et al., 2015), and it is likely that this represents a species complex. *Nebela penardi* has only been recorded in New Zealand and has a similar morphology to *Apodera vas*, however it has a characteristic keel first recognised by Brehm (1928) (Fig. 7-a3-a.4). This species occurs throughout New Zealand but is restricted to wetlands. *Certesella certesi* has a broad latitudinal distribution and is frequent in temperature forest soils and peatlands. *Certesella martiali* is rarer and appears to have a medium latitudinal distribution across New Zealand and has only been found in wetlands. We also observed a morphotype within the genus *Certesella* that has unique characteristics such as a bulbous neck with a notable constriction at the base of the neck (Fig. 7f.2). This is the first study to present this morphotype, which we call *Certesella* sp. a (Fig. 7f.2-f.3 and Supplementary Table 1). This morphotype has large pores located in the main body towards the neck, which are larger in this morphotype compared to the other *Certesella* species. *Certesella* sp. a has a broad latitudinal distribution across New Zealand. Interestingly, it can be difficult to distinguish between the different morphotypes of *Certesella* as some tests show a gradient between the characteristics (Fig. 7).

Lucid key

The interactive multiple-entry key was created for the identification of testate amoebae to species and subspecies level in New Zealand. However, as 119/128 morphospecies and morphotypes in the Lucid Key are cosmopolitan, this key has widespread utility beyond New Zealand. In the Lucid Key, there is a list of 17 features (different categories of test morphological characteristics) divided into 56 states (Table 2). To ease the process, the most taxonomically important and easily observable features were placed at the top of the list (for example, number of apertures was listed first, and position of aperture was second). Just over 60% of morphospecies/morphotypes can be identified within five or less steps and 75% can be identified in eight or less steps using the Lucid Key.

Discussion

Synthesised database

Prior to this investigation, a total of 98 testate amoebae morphotypes had been reported from New Zealand. The number is now 128, classified into 107 morphospecies within 37 genera. While this is a notable increase in the number of morphospecies/morphotypes recorded in New Zealand, the total number is still relatively low compared

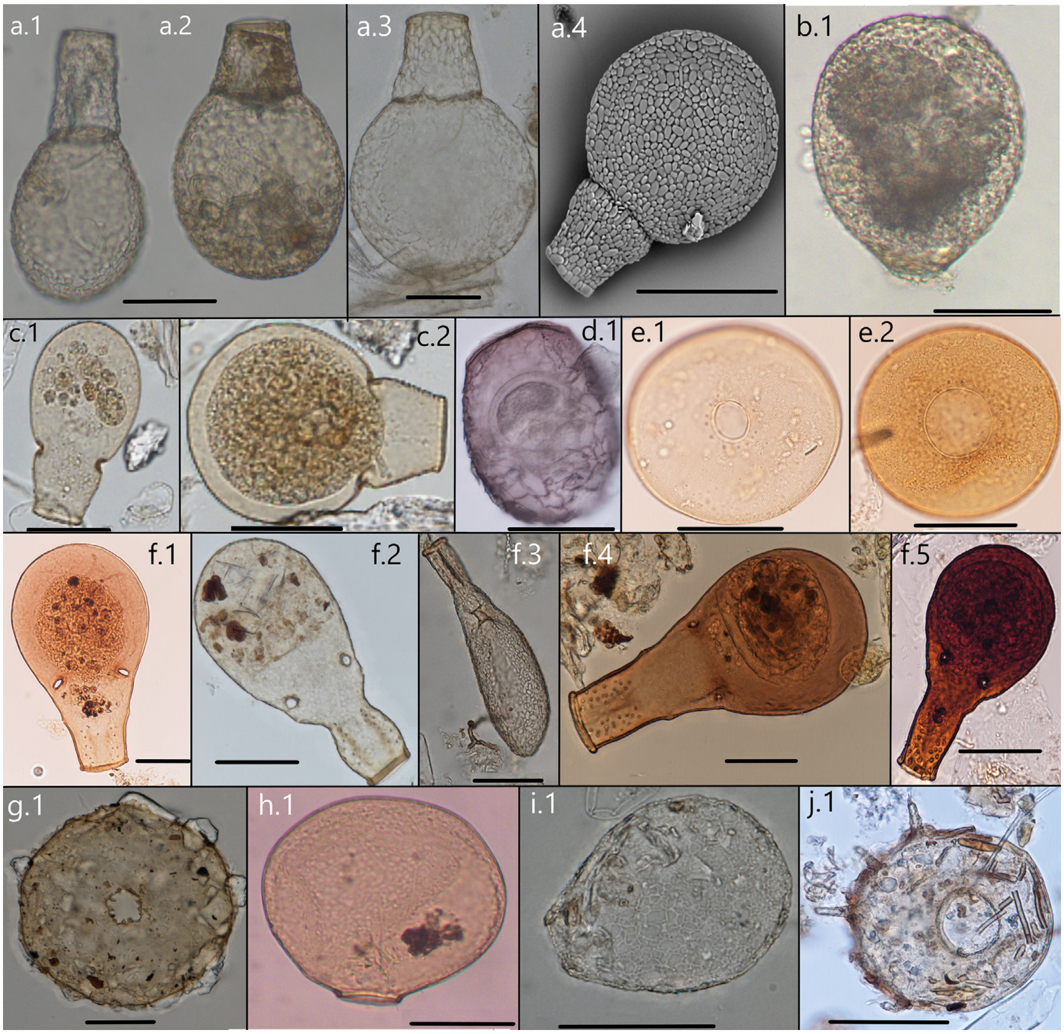

Fig. 7. Light micrographs and scanning electron micrographs of common, newly recorded, and endemic testate amoebae found in New Zealand peatlands: a. *Apodera vas* (Penard 1911): a.1 *A. vas* – narrow type, a.2 *A. vas* – broad type. a.3-4 *Nebela penardi* Brehm 1928. b.1 *Assulina scandinavica*. c. *Alocodera cockayni* (Penard 1911): c.1 *A. cockayni* – narrow type, c.2 *A. cockayni* – broad type. d.1 *Heleopera rosea*. e.1 *Arcella catinus*, e.2 *Arcella discoides*. f. *Certesella* (Penard 1911): f.1 *Certesella certesi*, f.2-3 *Certesella* sp. a in broad (f2) and lateral (f3) views, f.4-5 *Certesella martiali*. g.1 *Trigonopyxis microstoma*. h.1 *Nebela flabellulum*. i.1 *Argynnia dentistoma*. j.1 *Centropyxis aculeata*. Scale bar is 50 m in all images.

to other regions of the world. Smith, Bobrov, and Lara, (2007) collated information on testate amoebae diversity from around the world. Their list reaches 229 taxa for South and Central America, 428 for Australia, 648 for Africa, and 1031 for Europe. The higher number of testate amoebae in these regions may be due to the much larger land masses and broader range of sampled habitats and biomes. The number of testate amoebae recorded for smaller islands is notably less, with 65 taxa recorded for Îles Crozet, Southern Indian Ocean (Vincke et al., 2004), 24 for Livingston Island, Southern Ocean (Todorov and Golemansky, 1996), 43 for Amsterdam island, South Indian Ocean (Heger et al., 2009), 57 for Devon Island, Arctic Ocean (Beyens et al., 1990), 44 for peatlands in Santa Cruz, Galápagos, North-South Pacific Ocean (Fournier et al., 2016) and 45 for Guamblin Island, South Pacific Ocean (Fernándezet al., 2016).

Fernández et al. (2015) traced the evolution of species number recorded as a function of time throughout Chile and reached the conclusion that despite the high diversity (416 species recorded, an 81% increase on Smith et al. (2007)'s list) across the region, the true diversity is yet to be fully resolved. Smith et al. (2007) stressed that the higher diversity recorded for many regions may reflect the intensity of sampling effort rather than the true diversity. For example, Europe has been the focus of research for over two centuries and it is thus logical that this is where the highest total number of 1031 species was reported. New Zealand, although a much smaller landmass, is clearly still strongly under-sampled and all biomes and geographical regions

Table 2. List of characters and states to be selected for testate amoebae identification using the Lucid Key platform.

Features	State level 1	State level 2	State level 3
Number of apertures	● 1 ● 2		
Position of aperture	● terminal ● sub-terminal / off-central ● ventral		
Test shape	● spherical ● ovoid ● cylindrical/oblong ● pyroid/tear-shaped ● piriform ● flask-shaped ● irregular		
Composition of test	● test made of secreted biosilica plates/scales (idiosomes)	● plates/scales regular	● plates/scales overlapping ● plates/scales not overlapping ● external material (xenosomes) attached to test
		● plates/scales irregular	● plates/scales overlapping ● plates/scales not overlapping ● external material (xenosomes) attached to test
	● test is proteinaceous	● smooth structure ● surface pattern apparent ● external material (xenosomes) attached to test	
	● test fully built from external material (xenosomes)		
Lateral pores present	● yes ● no		
Keel present	● yes ● no		
Test colour	● colourless ● greenish-grey to dark grey ● pale yellow to dark brown ● rose red/orange to violet		
Structure of aperture	● smooth ● uneven or undulating ● plates/scales present and are the same as body ● plates/scales present and ● are different from body ● teeth present ● particles present		
Aperture collar/lip	● present ● absent		
Shape of aperture	● straight ● slit like ● invaginated ● triangular ● irregular ● crenulated-toothed vcurved		
Length	● enter measurement		
Width	● enter measurement		
Diameter (if round)	● enter measurement		
Aperture width	● enter measurement		
Horns present	● yes ● no		

Spines	• restricted to posterior • randomly scatterend • restricted to side • absent
Neck constricted	• yes • no

have yet to be comprehensively sampled for testate amoebae (Table 1). Thus, it is expected that the knowledge of recorded testate amoebae will continue to rise as more research is carried out across different habitats and biomes, and also in understudied geographic regions, such as the North Island, and offshore islands close to the mainland (i.e., Stewart Island), along with the NZ subantarctic islands (such as the peat covered Snares Islands, Auckland Islands and Campbell Island).

Testate amoebae richness, diversity, and biogeography

Wetlands have been the most intensively sampled habitat for testate amoebae, and the recorded number of species is three times higher than that of temperate forest soils and litters (Bamforth, 2015) or *Sphagnum* (Hoogenraad and De Groot, 1948). The diversity of testate amoebae was much higher in wetlands from the West Coast and Southland regions of the South Island, where most of the samples were collected. Grassland pastures have been largely undersampled with only 16 recorded morphospecies/morphotypes (Bamforth, 2015; Stout, 1984). The testate amoebae community from aquatic habitats is also largely unknown.

The earliest systematic recording of testate amoeba communities from New Zealand wetlands (specifically ombrotrophic bogs) was undertaken by Charman (1997), McGlone and Wilmshurst (1999), Wilmshurst et al. (2002), and Wilmshurst et al. (2003). This was carried out across 19 sites spanning the North and South Islands. Of these, 17 sites (55 samples) were in the South Island and only two sites (eight samples) from the North Island. The testate amoebae data presented for the first time in this study, were obtained from 33 sites, 27 (125 samples) from the South Island and six (32 samples) from the North Island.

The reason for this sampling bias and greater diversity of testates in the South Island is in part due to the comparatively greater number of West Coast in the South Island, many of which have generally higher ecological integrity (particularly in West Coast) (Ausseil et al., 2008) and show better test preservation (e.g., Hazell, 2004). It is estimated that 10% of the New Zealand mainland was covered by wetlands before human arrival around 780 cal. yr BP (Ausseil et al., 2011; McGlone, 2009). Although early indigenous Māori settlers caused extensive deforestation of dryland forests by burning (Argiriadis et al., 2018; McWethy et al., 2010; Perry et al., 2014), wetland extent was minimally affected until after European arrival (1800s) when wetland loss accelerated rapidly for development and agriculture (McGlone, 2009), reaching a ca. 90% loss of original extent today (Ausseil et al., 2011). Wetlands in the North Island have been more extensively drained since European contact (McGlone, 2009) and suffer varying degrees of degradation as a result of drainage, fragmentation and area loss (e.g., Robertson et al., 2019). As wetland destruction is ongoing, there is a clear risk that some yet to be described testate amoebae and other microscopic organisms are threatened in New Zealand. Furthermore, if an unknown proportion of these potential new taxa are endemic, they may be globally threatened. The conservation of microbial diversity is generally not a conservation priority but should be considered for the ecological functions they play as well as for the preservation of unique diversity (Cotterill et al., 2008).

Overall, there was higher diversity in Amoebozoan testate amoebae (86 taxa in total) compared to the SAR supergroup (33 taxa in total) in New Zealand wetlands (Fig. 4b). Amoebozoan testate amoebae include cosmopolitan taxa of both large (such as *Nebela* spp., *Centropyxis* spp., *Heleopera* spp.) and small (such as *Difflugia pulex*, *D. pristis*, *Phryganella paradoxa*, *Cryptodifflugia oviformis*, *Pseudodifflugia* spp.) size classes. SAR testate amoebae include a high number of small cosmopolitan taxa (e.g. in genera *Trinema*, *Sphenoderia*, *Corythion*, and *Euglypha*) and some larger taxa (e.g. in genera *Euglypha, Assulina* and *Placocista*). The higher number of Amoebozoan testate amoebae was also highlighted in Chile by Fernández et al. (2015), where this was attributed to the fact that many testate amoebae in the SAR group are smaller and harder to see under the microscope. Swindles et al. (2020) observed that many members of the SAR group have weak idiosomic siliceous tests (species of the genera *Euglypha, Corythion, Sphenoderia, Trinema* and *Tracheleuglypha*) and these are indeed often poorly preserved in older peats (Mitchell et al., 2008; Wilmshurst et al., 2003). Swindles and Roe (2007) and Payne (2007) showed that these testate amoebae were particularly vulnerable to test decomposition due to low pH conditions or through desiccation. As most NZ wetlands sampled for testate amoebae were ombrotrophic peatlands, which have a notably low pH, this result is not surprising. With the future inclusion of samples from other habitats, it is expected that the number of testate amoebae in the SAR group will increase.

A notably high number of small (<45 µm) taxa have also been recorded in both the SAR and Amoebozoa groups in New Zealand wetlands by Charman (1997), McGlone and Wilmshurst (1999), Wilmshurst et al. (2002), Hazell (2004) and McKeown et al. (2019). Moreover, Bamforth (2015) states that these smaller taxa (most notably *Cryptodifflugia*, *Pseudodifflugia* spp., *E. rotunda*, *Trinema lineare*, *Trinema enchelys*, *C. dubium* and *Assulina muscorum*) comprise 15–30% of the testate amoeba community in New Zealand temperate forest soils and litter. Wanner and Xylander (2005) suggest that these small taxa are among the first protists to appear in disturbed forest habitats. The relatively large proportion of small testate amoebae recorded in samples from NZ wetlands and forest habitats is yet to be fully explored. We speculate that the strong representation of small tests may be due to their small size allowing them to be distributed (potentially by wind) more easily than larger testate amoebae and may explain why these small tests appear first in disturbed forest soils. We note that several studies have recently shown that testate amoebae have morphological and physiological traits derived through evolutionary adaptation to ecosystem processes and assembly mechanisms (Fournier et al., 2015; Jassey et al., 2015; Koenig et al., 2018; Krashevska et al., 2020; Marcisz et al., 2016), it could well be that smaller testate amoebae are more numerous due to functional adaptation. Obviously, further research would help to explain the diversity and ecology of small testate amoebae in NZ, but this is beyond the scope of this paper.

Our study shows that 50% of testate amoeba morphospecies and morphotypes are widespread across the New Zealand mainland, while 30% are restricted to the South Island of New Zealand. Such taxa include many *Centropyxis* spp. (*C. aculeata*, *C. aerophila aerophila*, *C. aerophila sphagnicola*, *C. orbicularis*, *C. sylvatica*, *C. minuta*) and *Difflugia* spp.. (*D. acuminata*, *D. bacillariarum*, *D. bacillifera*, *D. brevicolla*, *D. bryophila*, *D. elegans*, *D. globulosa*, *D. lucida*, *D. rubescens*, *D. tuberculata*, *D. carinata*). Other morphospecies which may be restricted to the South Island of New Zealand include *Nebela minor*, *N. subsphaerica*, *N. flabellulum*, *Heleopea rosea*, *H. petricola var amethystea*, *Assulina scandinavica* and *Euglypha scutigera*. Future research focusing on different habitats outside of the South Island will allow the latitudinal distribution of the species to be further refined.

Testate amoebae exhibiting restricted geographical distributions make up around 15% of morphospecies and morphotypes (Figs. 5b and 6; Supplementary Table 2). These include *Arcella rotundata*, *Argynnia vitrea*, *Cryptodifflugia sacculus*, *Pseudodifflugia patella*, *Difflugia lanceolata*, *D. humulis*, *Nebela barbata*, *N. penardiana* and *Trinema galeata*. These taxa are rare and found at a relatively low abundance in NZ wetlands. However, *C. sacculus, P. patella* and *T. galeata* are only found in grassland pastures (Stout, 1984) and more work is needed to determine if these morphospecies are restricted to this habitat. The distribution of the remaining 5% of morphospecies and morphotypes is unknown as this information was missing in the literature.

Three taxa are known only from sub-fossil samples: *Cryptodifflugia compressa*, *Hyalosphenia minuta* and *H. papilio*. *Cryptodifflugia compressa* tests were found downcore in Kopouatai and Moanatuatua bogs in the Waikato region of the North Island, while *H. minuta* and *H. papilio* were only found downcore in Kopouatai (Hazell, 2004). However, we consider the finding of *H. papilio* unlikely given its known geographical distribution as a complex of cryptic/pseudocryptic species restricted to the Holarctic realm (Singer et al., 2019). Thus, this record deserves to be checked and this species is not included in the New Zealand checklist.

Gondwana-specific testate amoebae

Whilst many New Zealand testate amoebae appear to be cosmopolitan in distribution, some of the larger morphospecies and morphotypes (>100 µm) are known to be geographically restricted to landmasses below the Tropic of Cancer (Smith et al., 2007). *Apodera vas, Alocodera cockayni*, *Certesella certesi* and *C. martiali* are the most frequently recorded geographically limited species and it has been suggested that they have ancient origins to the palaeo-continent of Gondwana (Smith and Wilkinson, 2007). *Apodera vas* is the most well-documented species proven to be restricted almost exclusively to the Gondwana continents and subantarctic islands. It has been recorded throughout the Southern Hemisphere, including New Zealand (Charman, 1997; Hoogenraad and De Groot, 1948; McGlone and Wilmshurst, 1999; McKeown et al., 2019; Wilmshurst et al., 2002, 2003), Australia (Meisterfeld and Tan, 1998; Richters, 1907), South Africa (Gericke, 1932), Marion Island (Grospietsch, 1971), Îles Crozet (Vincke et al., 2004, 2006) and South Georgia Island (Beyens et al., 1995; Smith, 1982). *Apodera vas* has also been reported from the lower latitudes in the Northern Hemisphere below the Tropic of Cancer, such as West Africa (Bonnet, 1978; Golemansky, 1963), East Africa (Mitchell and Meisterfeld, 2005), Sumatra (Hoogenraad and De Groot, 1940), Nepal (Bonnet, 1977) (but possibly rather a *Padaungiella*, Bonnet pers. com.), and Central America (Laminger, 1973).

Our morphological analysis of *A. vas* suggests there are two different morphotypes found in New Zealand (Fig. 7-a.1-2). Penard had already noted in the very first publication on New Zealand testate amoebae the existence of two morphotypes in *Apodera* (*Nebela*) *vas*, a “normal” type 160–165 µm in length and a smaller type 95 µm in length (Penard, 1911). Thus, it was the size rather than the shape that differed in this case. Morphological variability is also observed in *Alocodera cockayni*, *Certesella* spp. along with *Nebela penardi* (similar morphology to *Apodera vas* but with a distinctive keel) in New Zealand (Fig. 7a.3-4). *Alocodera cockayni* appears to have two morphotypes that have not been identified before this study, one is wider with a shorter neck (*A. cockayni* – broad type) compared to the other (*A. cockayni* – narrow type) (Fig. 7c.1-c.2). *Certesella* spp. (including *C. certesi*, *C. martiali*, *C.* sp. a) all exhibit high morphological variability and at times it can be difficult to distinguish between the different morphotypes of *Certesella* as some tests show a gradient between characteristics (Fig. 7f.1-f.4). The morphological variability of *A. vas*, *A. cockayni* and *Certesella* spp. in New Zealand is also noted in Chile and Argentina (Zapata et al., 2008; Zapata and Fernández, 2008; Fernández et al., 2015). This may suggest (1) these are related morphospecies/morphotypes or (2) several pseudo-cryptic morphospecies/morphotypes share an ancient lineage but have evolved to be endemic to their current biogeographical region. Future biometrical and phylogeographic research is needed to assess the full diversity within *Apodera*, *Alocodera* and *Certesella* to determine if the high morphological variability evident in these genera (across New Zealand, Chile and Argentina, and even Australia and Antarctica) are indeed new species that evolved in isolation or are in fact more closely related.

Testate amoebae – colonisation to New Zealand

The origin and age of New Zealand terrestrial biota has been the focus of strong debates with claims for ancient vicariance (Heads, 2009) to arguments that suggest the entire biota is from long-distance dispersal (Landis et al., 2008). The emerging consensus eludes to a complex history of colonization for the formation of the New Zealand biota, with some taxa showing Gondwanan lineages (Buckley et al., 2020; Wallis and Jorge, 2018; Muirienne et al., 2014). Much biogeographic debate has been driven by phylogenetic data from vertebrates (Worthy et al., 2017) and plants (Heenan and McGlone, 2019), but with a growing number of studies on invertebrates (Buckley et al., 2015, 2020). However, microorganisms such as testate amoebae have the potential to provide another perspective on the origin of NZ biota. Phylogenetic analyses of *Apodera vas*, *Alocodera cockayni*, *Nebela penardi* and *Certesella* spp. may be able to elucidate the origin of these taxa. The broad distribution of most of these taxa across the Southern Hemisphere suggests that they do disperse, but a vicariant origin for at least some of New Zealand testate amoebae (e.g. *Nebela penardi*) may be possible. Following the breakaway of the Zealandia landmass from Gondwana ca. 80 million years ago, survival of the terrestrial descendants of this Gondwana biota require that at least some of the NZ landmass remained above sea level since the rifting event. Oligocene drowning is thought to have reduced Zealandia to approximately 18% of its current size (Cooper and Cooper, 1995) during maximum marine transgression (Mildenhall et al., 2014). Heenan and McGlone (2013) argue that this undoubtedly would have led to greater habitat heterogeneity and likely more opportunity for speciation. Testate amoebae offer an excellent candidate group to weigh in on the debate as *Apodera vas*, *Alocodera cockayni*, *Nebela penardi* and *Certesella* spp. are some of the largest morphospecies/morphotypes in our checklist with a well-defined restricted geographical distribution to below the Tropic of Cancer. Studies by Wilkinson (2001) and Yanget al. (2010) observe that testate amoebae smaller than 100–150 µm in length have a limited geographic distribution, suggesting a lower potential for wind dispersal. *Apodera vas* is the flagship species for a Gondwana origin, yet is also found on isolated oceanic islands, suggesting that although rare, long-distance dispersal is possible (e.g., attached to migrating birds). However, the somewhat larger and highly conspicuous *Nebela penardi* has only been reported from New Zealand, which could suggest that it evolved from *Apodera vas* following its separation from Gondwana. If true, then this would mean that testate amoebae include examples for both vicariance and long-distance dispersal. Future research, especially a phylogeographical study and the development of a molecular clock are needed to clarify this idea.

Furthermore during the Pleistocene epoch, New Zealand underwent repeated cycles of glaciation, with glaciers covering up to 30% of the South Island landmass (Carter, 2005; Wood et al., 2017). The large climate fluctuations during this time would have altered habitats suitable for testate amoebae to thrive. However, large portions of the South Island (namely Marlborough, part of Otago, and Southland) and the North Island were not covered with glaciers (Wallis and Trewick, 2009) during the last glacial maximum. This may have allowed these areas to be important refugia for endemic or Gondwanan testate amoebae. As the climate warmed into the Holocene, forest cover and wetlands expanded (McGlone, 2009), providing extensive suitable habitats for the re-establishment of testate amoebae from potential refugia. *Apodera vas* (both morphotypes), *Alocodera cockayni* – narrow type, *C. certesi*, *C. martialli*, and *Certesella* sp. a are all found in New Zealand wetlands (specifically bogs). *Apodera vas, C. certesi* and *A. cockayni* - narrow type have also been recorded in peatlands, forest litter, soils and *Sphagnum* across New Zealand, and in South Island Holocene peat cores (along with many other taxa) dating back to c. 8500 calibrated years before present (Wilmshurst et al., 2003). None of the above taxa have been recorded in grassland or pasture and they are unlikely to occur in such habitats.

Cosmopolitan taxa and recent colonisation

Morphospecies in New Zealand that appear to exhibit global distributions include the cosmopolitan *Argynnia caudata* and *A. dentistoma*, *Nebela collaris*-complex, *N. tincta*, *Padaungiella lageniformis*, *P. tubulata, P. wailesi*, *Centropyxis cassis*, *C. constricta* and *C. platystoma*, *Assulina muscorum*, *Amphitrema wrightianum*, *Euglypha* *filifera*, *E. strigosa*, *E. tuberculata*. Smaller taxa (<45 μm) also appear to present a broad global distribution, such as *Cryptodifflugia oviformis*, *Difflugia pristis*, *D. pulex*, *Pseudodifflugia fulva*, *Corythion dubium*, *Sphenoderia fissirostris*, *S. macrolepis, Euglypha laevis*, *E. rotunda*, *Tracheleuglypha dentata*, *Trinema complanatum*, *T. enchelys*, *T. lineare*. Many of these morphospecies are commonly found in wetlands across the world and are also abundant in New Zealand wetlands and Holocene peat records (McGlone and Wilmshurst, 1999; Wilmshurst et al., 2002, 2003). It is however likely that such supposedly cosmopolitan taxa contain several cryptic species differing in their distribution or ecology, as observed in several groups including both small taxa such as *Euglypha rotunda* (Wylezich et al., 2002) and larger taxa such as the *Nebela collaris* complex (Singer et al., 2015) and *Hyalosphenia papilio* (Heger et al., 2013; Singer et al., 2019). Such a pattern would agree with the idea of low probability long-distance dispersal combined with local speciation. Given the impact of human activities on New Zealand mostly since European colonisation and the drastic changes in biota and especially the introduction of numerous species to New Zealand, it also remains possible that some testate amoeba taxa have recently been introduced to New Zealand. One way to assess this would be to compare the diversity of living taxa with the diversity found preserved in peat and sediment cores. Another way would be to compare the genetic similarity of morphotypes found in New Zealand with those elsewhere globally, to rule out human dispersal (see Wilkinson, 2010).

Lucid key

Considering the number of testate amoeba morphospecies/morphotypes recorded in New Zealand (128 morphotypes, 107 morphospecies), the interactive key includes relatively few characters (17 features and 56 states, Table 2). This key includes all the testate amoebae recorded in the checklist and offers advantages over dichotomous identification keys, such as the ability to use subsets of characters, effective use of multi-state characteristics, freedom to follow more than a single pathway, and inclusion of media-rich information. In addition, this interactive online key provides a framework to build upon future identifications and changes to taxa with anticipated future molecular work. It can also be easily updated. This key will allow quicker and more accurate identification of testate amoeba morphospecies/morphotypes and faster morpho-phylogenetic updates.

Conclusion and future studies

This study updates the known testate amoebae of New Zealand to 128 morphotypes classified into 107 morphospecies within 37 genera and provides an accompanying freely-available Lucid key to aid with their rapid identification. Testate amoebae diversity for the region has notably increased with the greater amount of research through time. However, we are still far from understanding the true diversity and distribution of this group of organisms in the New Zealand context. Our analysis shows that testate amoebae are most diverse in wetlands from the South Island. However, this pattern is likely to be an artefact of uneven sampling effort, since other habitats have either been destroyed or are much less explored, and work has largely concentrated on South Island wetlands.

We suggest a priority for future studies is to aim for an improved understanding of testate amoeba diversity, ecology and habitat preferences across New Zealand. This will increase the power of this group of microorganisms to be used as valuable indicators of past and present environmental conditions. Future testate amoebae research would benefit from:

1. Focusing on geographical areas that are underexplored such as the North Island and offshore islands (e.g., Stewart Island, the NZ subantarctic Islands, and northern islands that have always been disconnected from the main islands by deep water).
2. Exploring other wetland types (i.e., marshes, fens, swamps, lakes, saltmarshes) along with other aquatic and terrestrial habitats. Such work would allow a more comprehensive understanding of testate amoebae species distribution and habitat preference.
3. Applying high throughput sequencing (metabarcoding or metagenomics) of environmental DNA (eDNA) to characterise New Zealand testate amoeba communities. The advantages of this approach include enabling more rapid, cost-effective and thorough sampling of testate amoebae diversity in soils compared to traditional microscope-based methods, although presently microscopy still provides better estimates of relative species abundances.
4. Conducting classical DNA barcoding studies combining morphological analyses and the sequencing of targeted barcoding markers (e.g. COI). Increasing application of eDNA in the study of testate amoeba communities requires significant expansion of current reference sequence databases, both in terms of taxonomic representation and the amount of genetic sequence data for each taxon, making this a key priority. Such work will also make it possible to infer phylogenetic relationships and explore phylogeographic patterns. Phylogenetic studies targeting specific taxa (*Apodera vas* and *Nebela penardi*) used in tandem with geological evidence has the potential to provide an insight into the origins and age of NZ testate amoeba taxa, which can in turn provide some additional evidence to the complex biogeographic history of New Zealand's biota.
5. Analysing more palaeoecological records from wetland and lake sediment cores to assess how testate amoebae assemblages have changed through time and responded to long term climate changes and human impacts. Palaeoecological records will also be able to detect potential species introductions, which would be the first such demonstration to our knowledge for microbial taxa. New Zealand is the ideal place to test such an approach as initial human settlement was relatively recent (Wilmshurst et al., 2008).

Acknowledgements

The authors would like to acknowledge all landowners and their permission to access the sites used for this research; Ngāi Tahu, Te Rarawa, and Ngāi Takoto Iwi at Tangonge, Ngāi Apakura Iwi at Moanatuatua, along with the private landowners and the Department of Conservation. We would also like to thank Ferry Siemensma for allowing us to use several testate amoeba images from the website https://www.arcella.nl/ and to Alex Fergus for his assistance with field work. This project was funded by a Smart Ideas grant (C09X1616) and the Strategic Science Investment Fund, both from the New Zealand Ministry of Business, Innovation and Employment's Science and Innovation Group. Finally, we would like to thank Leonardo D. Fernández and an anonymous reviewer for their constructive comments and suggestions, and for the time they devoted to review the article.

Funding statement

We acknowledge funding from The Ministry of Business, Innovation and Employment – Smart Ideas Programme (contract C09X1616: Wetland Assessment and Monitoring Tool (WAAM) – pre-human baselines for assessing, monitoring and restoring New Zealand's wetland ecosystems) and Strategic Science Investment Funding for Crown Research Institutes from the New Zealand Ministry of Business, Innovation and Employment's Science and Innovation Group. Leverhulme Trust grant RPG-2015-394 (Holocene evolution of the Southern Annular Mode using novel peat isotope proxies) funded data collection for unpublished samples that are included in the database. This study also benefited from the scientific leave of EADM in New Zealand during the academic year 2017–18.

Data resource

The data underpinning the Lucid Key reported in this paper is deposited in the Manaaki Whenua – Landcare Research Repository at https://keys.landcareresearch.co.nz/testateamoebae/.

Link to version 1 and associated date of key deployment at: https://keys.landcareresearch.co.nz/testateamoebae/version.txt.

We recommend following the ITP Lucid key best practice guide when using this key at http://idtools.org/lucid_best_practices.php.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2021.125789.

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