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Environmental Controls on Pore Number in *Hyalosphenia papilio*: Implications for Paleoenvironmental Reconstruction

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Summary. Testate amoebae are routinely used as paleoenvironmental indicators. However, considerable variability occurs in test morphology, even within commonly identified taxa. Relationships between morphological variability and environmental conditions might be useful in paleohydrological studies of peatlands, assuming good preservation of characteristics. *Hyalosphenia papilio* is a common taxon, well preserved in *Sphagnum* peatlands, that displays variability in the number of pores on the broad side of the test. We assessed whether variability in pore number was related to substrate moisture by comparing the abundance of individuals with different numbers of pores to measured water-table depths at 67 sites in North America. Results indicated that the abundance of individuals with higher numbers of pores increased in wetter conditions. Individuals with 2 pores were relatively widespread, although they dominated drier habitats. Transfer functions developed with and without pore-number quantification suggest that when communities contain abundant individuals with greater than 2 pores, water-table depth reconstructions can be improved by including pore-number information. Results have implications for peatland paleohydrological studies and suggest that greater exploitation of morphological variability could improve testate amoeba-based reconstructions of past environmental change.

Key words: Testate amoebae, *Hyalosphenia papilio*, *Hyalosphenia ovalis*, paleoecology, hydrology, peatlands.

INTRODUCTION

Testate amoebae are a polyphyletic group of shell-producing protists. Their shells, or tests, are morphologically distinct and often well preserved in peatland and lake sediments, making them useful indicators of past environmental conditions (Beyens and Meisterfeld 2001, Charman 2001, Mitchell *et al.* 2008). For example, testate amoebae are routinely used to reconstruct past hydrological changes in oligotrophic peatlands, providing important perspectives on past hydrological and climate variability (e.g. Booth *et al.* 2006, Charman *et al.* 2006). Peatland paleohydrological applications of testate amoebae are possible because community composition is typically controlled by surface-moisture conditions, allowing the development of transfer functions to infer past moisture variation from subfossil communities (e.g. Woodland *et al.* 1998; Bobrov *et al.* 1999; Mitchell *et al.* 1999; Lamentowicz and Mitchell 2005; Payne *et al.* 2006, 2008; Charman *et al.* 2007; Booth 2007; Swindles *et al.* 2009). However, a great deal of morphological variability occurs within many

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testate amoeba taxa, resulting in a variety of taxonomic approaches (Charman 1999). Although taxonomic uncertainty has been problematic for addressing biodiversity and biogeographical questions, morphological variability is often related to environmental conditions (e.g. Heal 1963, Wanner and Meisterfeld 1994, Bobrov *et al.* 1995, Wanner 1999, Booth 2001), suggesting that it could be exploited to improve community-based environmental reconstructions (Mitchell *et al.* 2008).

Hyalosphenia papilio is a species of testate amoeba with several characteristics that make it a good candidate for investigation of morphology-environment relationships that have potential paleoenvironmental application. For example, the taxon is widely distributed, abundant in *Sphagnum*-dominated peatlands, and generally well preserved and well represented in Holocene peat sequences (e.g. Booth *et al.* 2004, 2006). Also, *H. papilio* tests are easily identified, being oblong-oval or pyriform in lateral view and characterized by straight sides that taper toward the pseudostome (i.e. the mouth or primary opening of the test) (Fig. 1). Like other members of the genus, the tests are made solely of proteinaceous material. Several small pores characterize the border of the broad side of the test, each enclosed by a slight thickening of the shell. Most individuals have two pores located along the broad edge of the test (Fig. 1A). However, more than two pores are sometimes present (Figs 1B, C); for example, Leidy (1879) noted the presence of two to six pores and others have described 2–3 pores (Cash and Hopkinson 1909), 2–6 pores (Penard 1902), 2–8 pores (Jung 1936b) or simply mentioned the occurrence of multi-pored individuals (Jung 1936a). Some authors have classified individuals with more than two pores as a separate form, *H. papilio* forma *multiporifera* (Jung 1936a, Grospietsch 1965), and especially large individuals with between 2–12 or 3–13 pores have

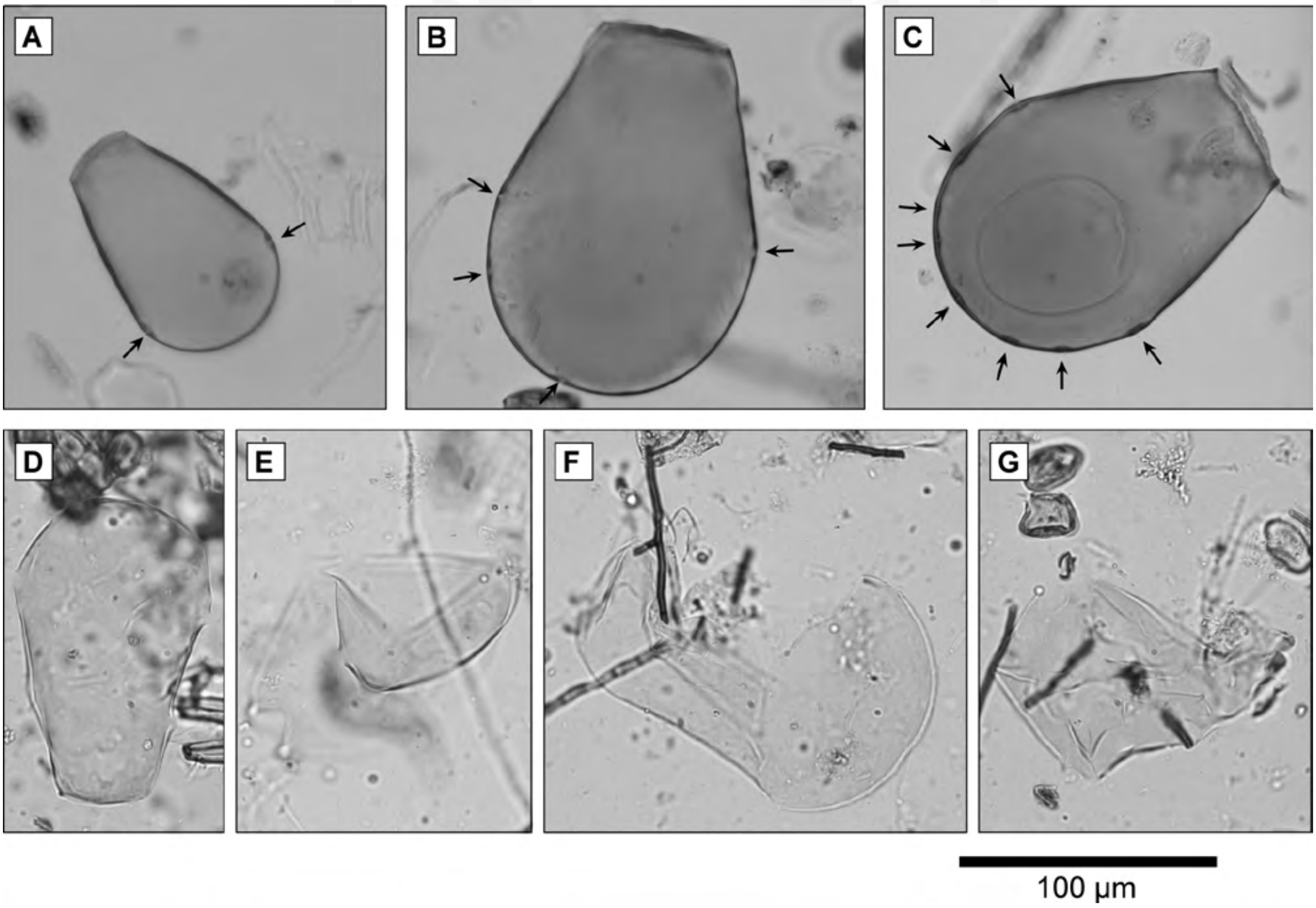

Fig. 1. Photomicrographs of modern (**A**–**C**) and subfossil Hyalosphenia papilio/ovalis (**D**–**G**). For modern specimens, arrows are shown indicating the number of pores present. Subfossil specimens were selected in an attempt to highlight the gradient of preservation typically encountered in oligotrophic peatlands. Scale bar is the same for all photomicrographs.

been described as a separate species, *H. ovalis* (Cash *et al.* 1919, Jung 1936b, Grospietsch 1965).

However, considerable confusion exists regarding the identification of *H. papilio* and *H. ovalis* in recent peatland studies. Although early descriptions separated *H. ovalis* from *H. papilio* by its larger size, more oval shape, and rounded keel, some recent peatland work has focused more on the pronounced convex tapering of the aperture in *H. ovalis* (Charman *et al.* 2000). However, using this criterion, tests identified as *H. ovalis* are generally smaller than *H. papilio,* which is inconsistent with early descriptions. In fact, even specimens of *H. ovalis* in Penard's slides at the British museum would be classified as *H. papilio* if the convex tapering of the aperture were used as the primary diagnostic feature (Charman *et al*. 2000). To add to the confusion, individuals of *Nebela tincta* sometimes lack plates, as is common in some modern samples and most fossil samples, yet these would be identified as *H. ovalis* using the approach of Charman *et al*. (2000). *N. tincta* is generally smaller than *H. papilio*, and confusion between these taxa may help explain the smaller size of tests identified as *H. ovalis* in recent studies (Charman *et al*. 2000). Recent studies in North America have not identified *H. ovalis* because of this confusion (e.g. Booth 2002, 2007; Markel *et al*. 2010). In these studies, tests consistent with *H. ovalis*, as described by Charman *et al.* (2000), have been identified as *N. tincta* and large tests consistent with the early descriptions of *H. ovalis* have been included within *H. papilio*. This broad definition of *H. papilio* is used in this present study, and would seem to be a better taxonomic treatment of the group (D. Charman, personal communication).

Some biometric characteristics of testate amoebae, such as test size, may be distorted by taphonomic processes, making these characteristics less useful for paleoenvironmental work. For example, qualitative observations suggest that *H. papilio* tests may become altered in highly decomposed peat deposits, with some subfossil individuals having thinner test walls and occasionally being enlarged and more transparent than modern tests (Booth, personal observation). Size can also be difficult to measure in tests that are crumpled or poorly preserved (Figs 1E–G). However, the number of pores is usually still discernable with careful microscopy, even in relatively poorly preserved tests. Therefore, if pore number is related to environmental conditions it could be used to provide paleoenvironmental information, and this might be particularly useful if applied in combination with community-based approaches. Since substrate moisture is a primary control on testate amoeba community composition in oligotrophic peatlands (e.g. Mitchell *et al.* 2008), and morphological variability in other taxa has been linked to available moisture (e.g. Heal 1963; Bobrov *et al.* 1995, 1999; Wanner 1999), we hypothesized that the number of pores in *H. papilio* was related to substrate moisture. We tested this hypothesis by conducting a comparative study of pore number and water-table depth at 67 sites within *Sphagnum*-dominated peatlands of North America. We used our data to highlight the potential paleoenvironmental application of pore number in *H. papilio*.

MATERIALS AND METHODS

Samples were collected as part of previous ecological investigations of testate amoebae in North America, and included testate amoeba communities from eastern North America (Booth 2002, 2007) and Alaska (Markel *et al.* 2010). Depth to the water table was measured at each site relative to the surface, with higher values indicating drier conditions and negative values indicating standing water. Testate amoebae were isolated from moss using standard methods (Hendon and Charman 1997). Samples with greater than 2% *H. papilio*, as a percentage of the total number of testate amoebae counted (minimum counted = 150) (Charman *et al.* 2000), were randomly selected from across the wetness gradient for this study. For each of these samples, the number of pores was counted on at least 50 individual *H. papilio* tests. The relative abundance of individuals with different numbers of pores was calculated in two ways; as a percentage of the 50 *H. papilio* tests examined and as a percentage of the total number of testate amoebae in the sample. To assess whether quantification of pore number affects the performance of paleoenvironmental reconstructions, transfer functions were developed with and without inclusion of pore-number morphotypes using a weighted average model. Standard jack-knifing (leave-one-out) methods were used to cross-validate transfer functions using C2 software (Birks 1998, Juggins 2003). Root mean square error of prediction (RMSEP) was used to compare the performance of transfer functions developed with and without pore quantification.

RESULTS AND DISCUSSION

Over 70% of the *H. papilio* individuals examined in this study had two pores (Fig. 2), confirming the dominance of this morphotype in *Sphagnum* peatlands (e.g. Leidy 1879). Individuals with 3, 4, 5, and 6 pores each made up less than 10% of the total number of *H. papilio*, and the abundance of individuals with more than 6 pores was even less (Fig. 2). Although *H. papilio* (or *H. ovalis*) individuals with 12 or 13 pores have been described previously (Cash *et al.* 1919, Jung 1936b, Grospietsch

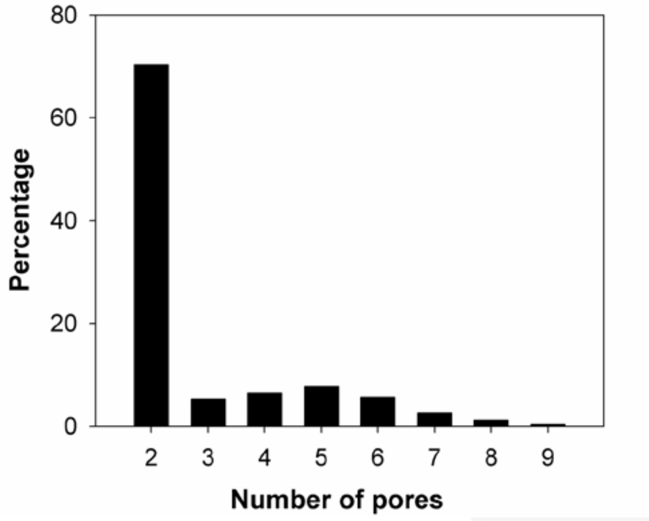

Fig. 2. Relative frequencies of of *Hyalosphenia papilio* with different numbers of pores (n = 3350).

1965), a maximum of nine pores was encountered in this study, indicating that the dataset did not capture the complete range of variability in the taxon.

Previous research has demonstrated that *H. papilio* occurs in highest abundance in moderately wet habitats within *Sphagnum*-dominated peatlands, although it has a relatively wide tolerance, occurring in shallow standing water as well as small hummocks (e.g. Woodland *et al.* 1998; Booth 2007, Swindles *et al.* 2009) (Fig. 3a). The samples analyzed as part of this study were selected to capture much of this moisture gradient; although at dry sites the number of individuals was insufficient (i.e. less than 2% of the total number of testate amoebae) to permit adequate quantification of pores (Fig. 3a).

In his original description of *H. papilio*, Leidy (1879) noted that the minute pores along the broad margin of the test probably permit the entry and departure of water in response to the contraction and expansion of the pseudopodia. Consistent with this observation, tests with larger volumes, such as those sometimes described as *H. ovalis*, often have more pores (Leidy 1879, Cash *et al.* 1919, Jung 1936b, Grospietsch 1965). Also, qualitative studies have suggested that individuals with greater than two pores occur more frequently in wetter habitats (Jung 1936a, b). Tests with more pores also are likely to lose more moisture in drier habitats, so may be less adapted to these conditions. Our results provide quantitative support for these early observations (Fig. 3b). Individuals with more pores are found in wetter habitats (Fig. 3b). In contrast, drier sites are nearly exclusively dominated by the 2-pore morphotype (Fig. 3b). However, although the 2-pore morphotype dominates drier habitats it also is commonly encountered along the entire moisture gradient. Even in wetter habitats the 2-pore morphotype is often still a relatively abundant form of *H. papilio* (Fig. 3b).

Although the widespread occurrence and abundance of the 2-pore morphotype limits the usefulness of using average-pore number as a direct indicator of water-table depth (Fig. 4), quantifying the abundance of each morphotype may be useful in paleoenvironmental studies. For example, the water-table depth optimum of each morphotype decreases with increasing pore number, from about 14 cm below the peat surface for the 2-pore type to about 6 cm of standing water for the 9-pore type (Fig. 3b). This range of difference in optima of the different morphotypes is about three times the mean error of transfer functions reported in many paleoenvironmental calibration studies (Woodland *et al.* 1998, Mitchell *et al.* 1999, Lamentowicz and Mitchell 2005, Payne *et al.* 2008, Charman *et al.* 2007, Booth 2007, Markel *et al.* 2010). Likewise, the tolerances of the multi-pored forms are narrower than the 2-pored form, suggesting that quantification of pore-number morphotypes may improve the performance of paleohydrological transfer functions.

However, jack-knifed validation of transfer functions developed with and without pore-number differentiation revealed only a small overall improvement in performance when the abundance of individuals with different numbers of pores was included (Table 1). The lack of significant improvement, even though different pore-number forms are clearly characterized by different water-table depth optima (Fig. 3b), was likely because the abundance of multi-pore types (i.e. > 2 pores), as a percentage of the total testate amoeba community, was often relatively low. Given the relatively low abundance of multi-pored forms in many samples, the performance

Table 1. Comparison of cross-validation statistics for weighted average transfer functions developed with and without pore-number differentiation of *H. papilio* (n = 67).

Transfer function	r^2	RMSEP (cm)
With pore-number differentiation	0.62	7.2
Without pore-number differentiation	0.61	7.3

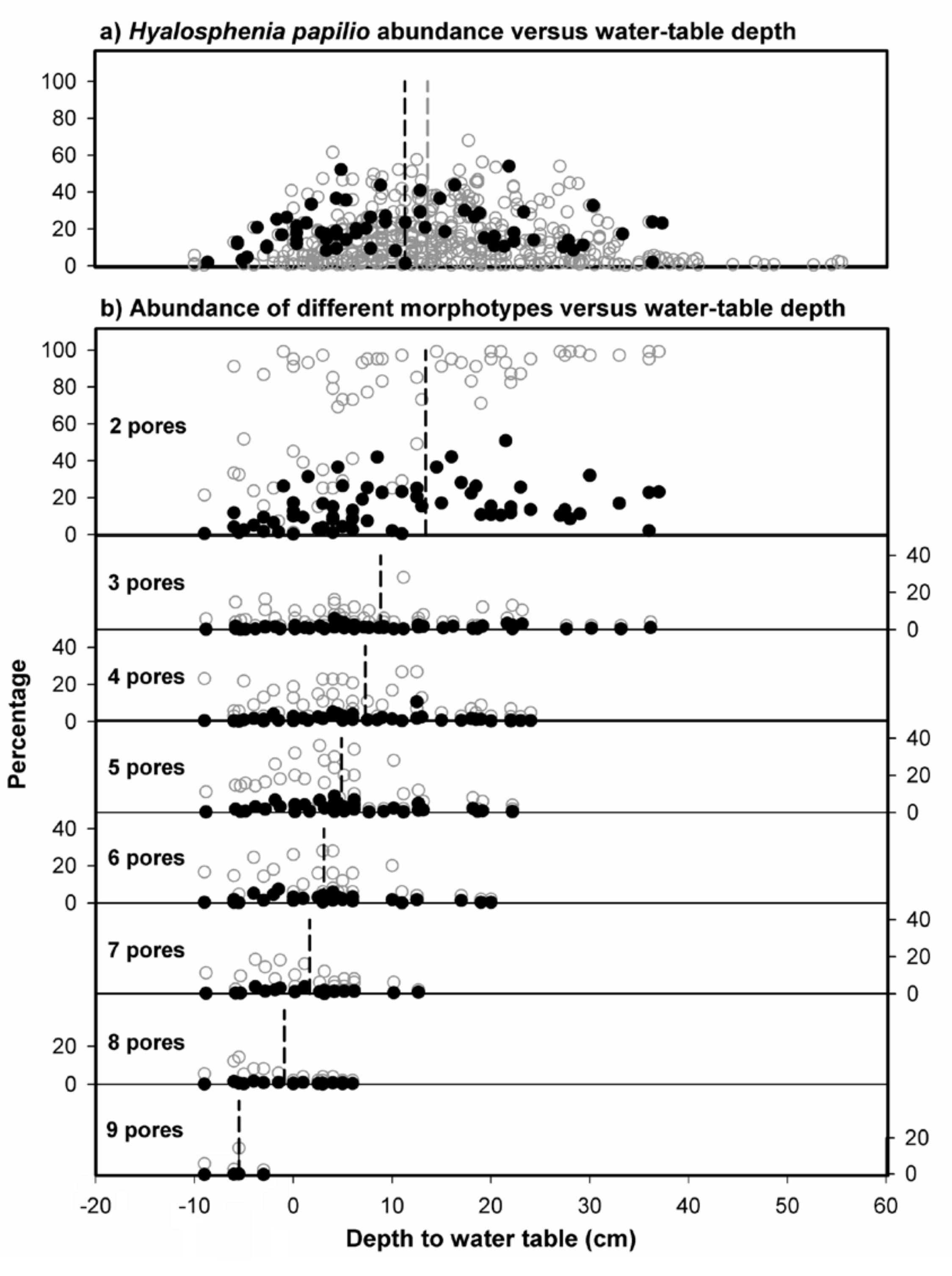

Fig. 3. Relationship between water-table depth and a) *Hyalosphenia papilio* abundance, expressed as a percentage of the total number of testate amoebae counted, and b) the abundance of *Hyalosphenia papilio* with different numbers of pores. Gray, open circles in (a) represent data from 850 sites in peatlands of North America (Booth 2002, 2007; Markel *et al.* 2010) and black, filled circles indicate the samples where the abundance of different pore-number morphologies was determined in the present study. The relative abundance of individuals with each pore number, expressed as a percentage of the total (n = 50) number of *H. papilio* individuals examined (gray, open circles) and as a percentage of the total number of testate amoebae counted (black, filled circles) are shown in (b). Dotted vertical lines indicate the optima water-table depth of each morphology, calculated using a weighted average.

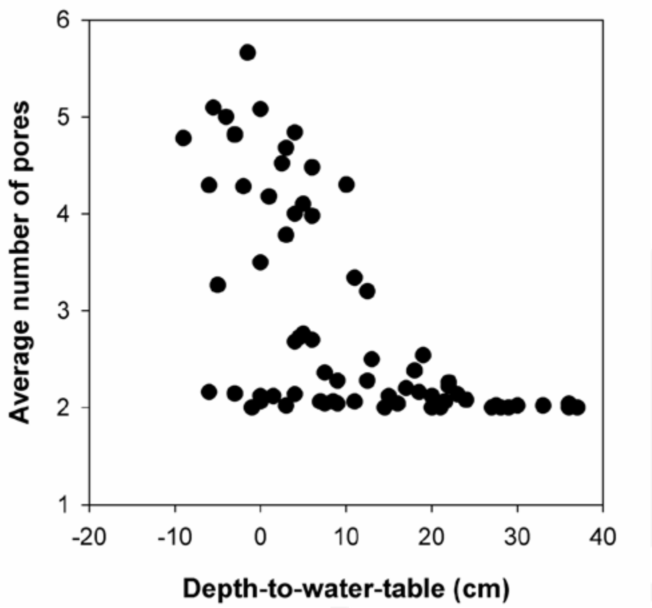

Fig. 4. Relationship between water-table depth and average pore number in *Hyalosphenia papilio*.

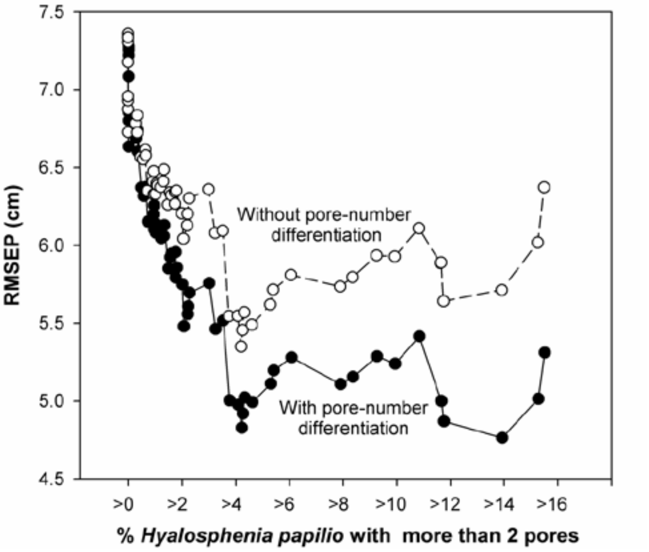

Fig. 5. Root mean square error of prediction (RMSEP) of transfer functions developed from the dataset, with and without the inclusion of the abundance of different pore numbers, for subsets of samples arranged according to the percentage of *Hyalosphenia papilio* individuals with more than two pores. Although the additional information on pore number does not substantially decrease the overall error of the transfer function (Table 1), improvement is greatest when the number of individuals with greater than two pores is high within the samples.

of the transfer functions was primarily determined by other more dominant taxa in the community. Consistent with this observation, pore-number quantification resulted in the greatest improvement in the performance of the transfer function in samples characterized by higher percentages of the multi-pored forms (Fig. 5). In fact, in samples where the abundance of multi-pored forms exceeded about 10% of the total testate amoeba community the RMSEP of the transfer function was improved by about 1-cm through the inclusion of pore-number quantification (Fig. 5). In samples with higher percentages of multi-pored forms, quantification of the abundance of pore-number morphotypes would likely result in even greater improvement in transfer-function performance.

Results of this study suggest that the separation of *H. papilio* into morphological forms based on the number of pores may provide more reliable paleoenvironmental reconstructions at sites and time periods where multi-pored forms of *H. papilio* are particularly abundant. In fact, given the large differences in water-table depth optima along the pore-number gradient (Fig. 3b), there is the potential for error in reconstructions if multi-pore forms dominate subfossil assemblages and pore-number differentiations are not made. Although the high amount of morphological variability in the testate amoebae has generally been a problem for ecological and biogeographical studies (Charman 1999, Heger *et al*. 2009), the results of this and other recent studies (e.g. Bobrov *et al*. 1995, 1999) highlight that this variability can be exploited to improve paleoenvironmental reconstructions. Further investigation of the environmental controls on test morphology, particularly morphological traits that remain unaltered in subfossil tests, would potentially lead to more reliable reconstructions of past environmental change.

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