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# A time-calibrated multi-gene phylogeny of the diatom genus *Pinnularia*

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ABSTRACT

*Pinnularia* is an ecologically important and species-rich genus of freshwater diatoms (Bacillariophyceae) showing considerable variation in frustule morphology. Interspecific evolutionary relationships were inferred for 36 *Pinnularia* taxa using a five-locus dataset. A range of fossil taxa, including newly discovered Middle Eocene forms of *Pinnularia*, was used to calibrate a relaxed molecular clock analysis and investigate temporal aspects of the genus' diversification. The multi-gene approach resulted in a well-resolved phylogeny of three major clades and several subclades that were frequently, but not universally, delimited by valve morphology. The genus *Caloneis* was not recovered as monophyletic, confirming that, as currently delimited, this genus is not evolutionarily meaningful and should be merged with *Pinnularia*. The *Pinnularia–Caloneis* complex is estimated to have diverged between the Upper Cretaceous and the early Eocene, implying a ghost range of at least 10 million year (Ma) in the fossil record.



## 1. Introduction

Diatoms are an extremely diverse group of unicellular algae that are uniquely characterized by a siliceous cell wall (the frustule) consisting of two valves and a number of girdle bands (Round et al., 1990) and a diplontic life cycle involving gradual size reduction during vegetative divisions and rapid size restitution, usually through sexual reproduction (Chepurnov et al., 2004). In the so-called pennate diatoms the valve pattern is organized bilaterally around the longitudinal axis, and in most cases the valve is elongate. Raphid pennate diatoms possess a pair of longitudinal slits along the apical axis (the raphe system) (Fig. 1), from which extracellular polymeric substances are exuded and used in locomotion and for adhesion to the substratum (Round et al., 1990). The raphe is considered a derived character state that distinguishes the raphid diatoms from the more ancestral araphid pennate forms that lack this structure and from the oldest known forms, the radially organized "centric" taxa (Sims et al., 2006). Based on fossil remains, the araphid pennates first appeared in the Upper Cretaceous (ca. 75 Ma; Chambers, 1966; Hajós and Stradner, 1975), and the raphe-bearing forms soon thereafter, around 70.6–55.8 Ma (Chacon-Baca et al., 2002; Pantocsek, 1889; Singh et al., 2006; Witt, 1886). Monophyly of pennate diatoms as a whole, as well as the raphid pennates, has been documented using SSU rDNA and *rbc*L sequences (e.g. Kooistra et al., 2003; Sorhannus, 2004, 2007). Since their origin, raphid pennate diatoms have diversified enormously and account for the majority of the over 200,000 extant species estimated to exist (Mann and Droop, 1996), indicating the evolutionary advantages conferred by the raphe (Sims et al., 2006).

Despite the diversity and ecological success of raphid pennate diatoms, relatively few detailed molecular phylogenetic reconstructions exist. Phylogenies applied at genus to ordinal levels have yielded partly unsupported taxon relationships (e.g. Bruder and Medlin, 2008; Trobajo et al., 2009), in part due to very limited taxon sampling and/or the use of a limited number of genetic markers (Mann and Evans, 2007). In addition, molecular phylogenies of individual genera have focused largely on the identification of

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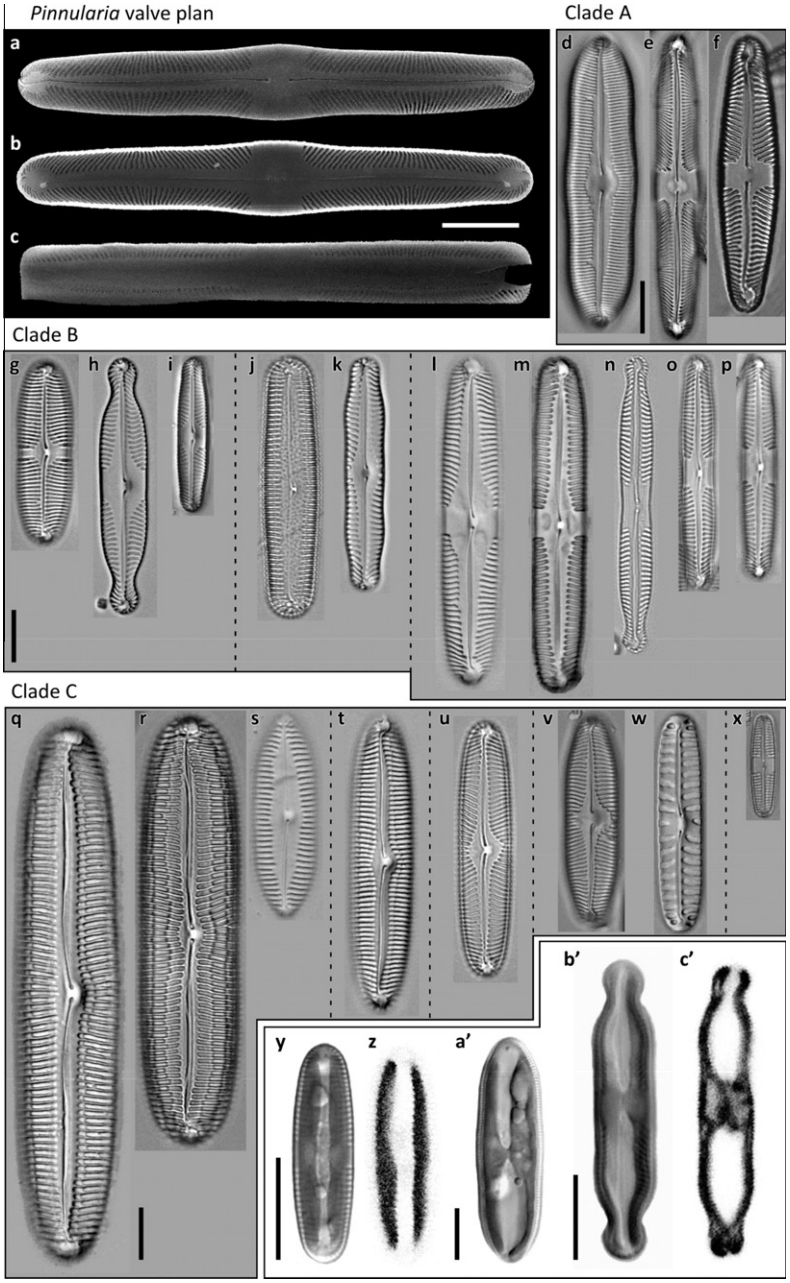


**Fig. 1.** Morphological variation in *Pinnularia* and representative illustrations of strains included in the multi-gene phylogeny. The valve construction of typical frustules belonging to the *P. divergens* group [strain (Tor7)c] is shown by scanning electron micrographs [outside (a), inside (b) and side (=girdle) view (c)]. Cultured and sequenced strains are illustrated by a series of light micrographs (d–x), divided, where appropriate, into subclades recovered in the phylogeny with vertical dashed lines. Clade A (d–f) includes *Caloneis lauta* (d) as well as *P. divergens* grade representatives (Tor7)c in (e) and (Tor1)b in (f). Clade B (g–p) includes the "*grunowii*" subclade (g–i) with *Pinnularia sp.* (Tor4)i in (g), *P. subanglica* Pin650 in (h), and *P.* cf. *marchica* (Ecrins4)a in (i); the "*nodosa*" subclade (j–k) with *P. acrosphaeria* (Val1)b in (j) and *P. nodosa* Pin885 in (k); and the "*subgibba*" subclade (l–p) represented by *P. parvulissima* Pin887 in (l), *Pinnularia* sp. "*gibba*-group" (Tor7)f in (m), *P. subcapitata* var. *elongata* (Wie)c in (n), *P.* sp. (Tor4)r in (o), and *Pinnularia* sp. "*gibba*-group" (Tor8)b in (p). Clade C (q–x) comprises the "*viridis*" subclade (q–r) represented by *P. neglectiformis* Pin706 in (q) and *P. viridiformis* (Enc2)a in (r); with its sister *P. acuminata* Pin876 in (s); the "*subcommutata*" subclade (t) represented by *P. subcommutata* var. *nonfasciata* Corsea10 in (t); forms that do not readily fit into well-defined subclades represented by *P.* sp (Wie)a in (u); the "*borealis-microstauron*" subclade (v–w) including *P.* cf. *microstauron* (B2)c in (v) and *P. borealis* Alka1 in (w); and subclade C1 (x) represented by *P.* cf. *altiplanensis* (Tor11)b in (x). Live cells of *Pinnularia sensu lato* (*i.e.* including *Caloneis*) also show two distinct plastid arrangements, which are illustrated by light (y, a′, and b′) and laser-scanning confocal microscopy of the autofluorescent organelles (z and c′). For example, representatives of the *P. subcommutata* and *gibba* taxa have parallel plastids on either side of the apical axis (y, z), while *Caloneis silicula* (a′) and *P. grunowii* (b′ and c′) have plastids that are joined by a central bridge. See text for details. All scale bars are 10 μm, and images (d–x) are reproduced at the same magnification to facilitate size comparisons between taxa.

cryptic diversity rather than the elucidation of evolutionary relationships between lineages (e.g. Beszteri et al., 2007; Evans et al., 2008; Lundholm et al., 2006). There are a few explicit time-calibrated phylogenies at the ordinal level (e.g. Medlin et al., 1996), the diatoms as a whole (e.g. Kooistra and Medlin, 1996; Medlin et al., 1997a; Sorhannus, 2007), the wider heterokont group (e.g. Brown and Sorhannus, 2010; Medlin et al., 1997b), or even eukaryotes (e.g. Berney and Pawlowski, 2006), but there are none at the generic level; furthermore, few analyses formalize the evolutionary associations between the timing of lineage splitting and ecological, morphological, physiological and/or reproductive strategies, life cycles and geographical distributions (but see Casteleyn et al., 2010). Furthermore, despite important recent micropaleontological discoveries, some of which confirm that particular genera