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Hatena arenicola gen. et sp. nov., a Katablepharid Undergoing Probable Plastid Acquisition

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***Hatena arenicola* gen. et sp. nov., an enigmatic flagellate of the katablepharids, is described. It shows ultrastructural affinities to the katablepharids, including large and small ejectisomes, cell covering, and a feeding apparatus. Although molecular phylogenies of the 18S ribosomal DNA support its classification into the katablepharids, the cell is characterized by a dorsiventrally compressed cell shape and a crawling motion, both of which are unusual within this group. The most distinctive feature of *Hatena arenicola* is that it harbors a *Nephroselmis* symbiont. This symbiosis is distinct from previously reported cases of ongoing symbiosis in that the symbiont plastid is selectively enlarged, while other structures such as the mitochondria, Golgi body, cytoskeleton, and endomembrane system are degraded; the host and symbiont have developed a morphological association, i.e., the eyespot of the symbiont is always at the cell apex of *Hatena arenicola*; and only one daughter cell inherits the symbiont during cell division, resulting in a symbiont-bearing green cell and a symbiont-lacking colorless cell. Interestingly, the colorless cells have a feeding apparatus that corresponds to the location of the eyespot in symbiont-bearing cells, and they are able to feed on prey cells. This indicates that the morphology of the host depends on the presence or absence of the symbiont. These observations suggest that *Hatena arenicola* has a unique "half-plant, half-predator" life cycle; one cell divides into an autotrophic cell possessing a symbiotic *Nephroselmis* species, and a symbiont-lacking colorless cell, which later develops a feeding apparatus de novo. The evolutionary implications of *Hatena arenicola* as an intermediate step in plastid acquisition are discussed in the context of other examples of ongoing endosymbioses in dinoflagellates.**

Key words: *Hatena arenicola*; Katablepharidophyta/Kathablepharida; *Nephroselmis* symbiont; plant evolution; plastid acquisition via secondary endosymbiosis; ultrastructure.

Abbreviations: EM = electron microscopy; ER = endoplasmic reticulum; ICBN = International Code of Botanical Nomenclature; ICZN = International Code of Zoological Nomenclature; LM = light microscopy; SEM = scanning electron microscopy; SSU rDNA = small subunit ribosomal DNA; TEM = transmission electron microscopy.

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Introduction

Eukaryotes are currently classified into five or six supergroups (Baldauf et al. 2000; Baldauf 2003; Bapteste et al. 2002; Nozaki et al. 2003; Simpson and Roger 2002), and eukaryotic autotrophs (e.g., plants and algae) randomly scatter across those supergroups. Eukaryotic autotrophs comprise nine distinct divisions in cell architecture, and this enormous diversity is explained by several endosymbiotic events (Bhattacharya et al. 2004; Falkowski et al. 2004; McFadden 2001). It is widely accepted that a primary endosymbiosis between a eukaryote and a cyanobacterial symbiont gave rise to the three extant primary eukaryotic autotrophs, Glaucophyta, Rhodophyta, and Viridiplantae (= land plants plus green algae) (see Marin et al. (2005) for an alternative primary endosymbiosis). Subsequently, secondary endosymbioses occurred between green or red algae and heterotrophic eukaryotic hosts. Two algal divisions (Euglenophyta and Chlorarachniophyta) acquired the plastids of green algae, while four algal divisions (Heterokontophyta, Haptophyta, Cryptophyta, and Dinophyta) and one parasitic phylum (Apicomplexa) acquired those of red algae (although some Dinophyta lost their original plastid and remained colorless or re-acquired different plastids as discussed below). An estimated two-thirds of today's algal diversity resulted from secondary endosymbioses (Falkowski et al. 2004; Graham and Wilcox 2000), and thus this process is important in understanding the evolutionary process of plant and algal diversification.

The transition of a symbiont to a plastid involves a series of changes in both the host and the symbiont (Cavalier-Smith 2003; Hashimoto 2005; van der Giezen et al. 2003), which include the establishment of a specific partner alga, lateral gene transfer from the symbiont to the host's nucleus (Katz 2002), the development of protein-transport machinery to carry proteins from the host cytoplasm to the symbiont (van Dooren et al. 2001), and synchronization of cell cycles so that the symbiont can be passed to host daughter cells during host cell division.

Evidence about plastid integration is accumulating (Andersson and Roger 2002; Archibald et al. 2003; Hackett et al. 2004b; Huang et al. 2003; Martin and Herrmann 1998; Martin et al. 2002; Martin 2003a, b; Nozaki et al. 2004; Stegemann et al. 2003), however, the intermediate steps in this process remain largely unknown. Some organisms appear to be in an intermediate stage of plastid acquisition, the best-known examples of which are the Cryptophyta and Chlorarachniophyta, whose plastids contain a vestige of the symbiont nucleus termed a nucleomorph (e.g. Douglas et al. 2001; Gilson et al., 2006). They are thought to represent a late stage of integration. Early stages of plastid acquisition can be found in the dinoflagellates (for reviews, Hackett et al. 2004a; Morden and Sherwood 2002; Schnepf and Elbrächter 1999), where the most dramatic changes are ongoing. The original plastids of dinoflagellates have been of red algal origin, though some dinoflagellates subsequently lost their original red-algal plastids, which were replaced by new ones via extra secondary or tertiary endosymbioses. These examples probably reflect stepwise changes in symbiotic conditions during integration (e.g. Hackett et al. 2004a), and are useful to understand the plastid acquisition process.

We discovered an undescribed flagellate, *Hatena arenicola* gen. et sp. nov., in October 2000, in an intertidal sandy beach in Japan. The organism appears to be in the process of plastid acquisition. Most cells of *H. arenicola* in the natural population have a green plastid-like structure with a red eyespot at the cell apex, though it is inherited by only one of the daughter cells during cytokinesis (Okamoto and Inouye 2005a). Molecular phylogenetic analysis of small subunit ribosomal DNA (SSU rDNA) and ultrastructural observations of the plastid-like structure reveal that it is not a plastid but an autotrophic endosymbiont belonging to the genus *Nephroselmis* Stein (Prasinophyceae, Viridiplantae). We previously reported the symbiotic nature of this association (Okamoto and Inouye 2005a). This paper describes the organism as a new genus and species of katablepharid, a group of flagellates recently designated the phylum Kathablepharida, division Katablepharidophyta (Okamoto and Inouye 2005b). We compare the symbiosis of *H. arenicola* with other examples of secondary symbioses in dinoflagellates to help elucidate the intermediate steps in the plastid acquisition process.

Results

Description

Hatena arenicola Okamoto et Inouye gen. et sp. nov.