to leave well-defined spore coat residues, while large-celled species such as *Bacillus cereus* and *Bacillus megaterium* may not. Lamana (1940a, 1954) studied modes of spore germination for nine species and found it to be of potential value for differentiation between the small-celled species and between this group and the large-celled species, but, with two exceptions (Burdon, 1956; Gould, 1962), little further attention has been paid to this character.

Bradley and Franklin (1958) showed that most of the 20 species they studied could be distinguished by electron microscopy of carbon replicas of spore surface patterns. Bulla et al. (1969) found that scanning electron microscopy gave inadequate resolution for such studies, but Murphy and Campbell (1969) achieved good resolution of *Bacillus polymyxa* spores by this method, and Gray and Hull (1971) considered this approach to be promising in the study of the *Bacillus circulans* complex. Later authors have sometimes described spore surface structure in proposals for new species, but too few such descriptions are available to judge the taxonomic value of spore surface characteristics across the genus.

Electron microscopy has revealed sword-shaped appendages radiating from one end of the exosporium of the spores of two phylloplane strains of *Bacillus cereus* (Mizuki et al., 1998). The proteinaceous spore appendages of 10 *Bacillus cereus* strains isolated from food-borne illness outbreaks and food industry sources showed some antigenic relationship, but when subjected to SDS-PAGE analysis none showed identical patterns (Stalheim and Granum, 2001). Smirnova et al. (1991) found that hemagglutination patterns of fimbriated *Bacillus thuringiensis* spores correlated with the subspecies of the strains rather than with their flagellar serovars. Song et al. (2000) reported that under strictly standardized growth conditions, spore fatty acid profiles, like those of vegetative cells, are stable and potentially of taxonomic value.

The microscopic morphologies of *Bacillus* species, especially of their sporangia, are well established as valuable characters. Smith et al. (1946, 1952) and Gordon et al. (1973) used cell size, appearance of cytoplasm and sporangial morphology as the basis of their division of the genus into three groups of species, and this arrangement still correlates quite well with the present classification of the aerobic endospore-formers. Sporangial morphology, and cell size, shape and cytoplasmic appearance, remain useful characters in polyphasic taxonomic studies, and sporangial characters are particularly valuable in identification. Spore shapes vary from cylindrical (Figure 10a) through ellipsoidal (Figure 10b–e, g) to spherical (Figure 10f); bean- or kidney-shaped, curved-cylindrical, and pear-shaped spores are also seen occasionally. Spores may be terminally (Figure 10f), subterminally (Figure 10a–g), paracentrally (Figure 10b, d, e, g) or centrally (Figure 10f) positioned within sporangia and may distend them (Figure 10c–f). Despite within-species and within-strain variation, sporangial morphologies tend to be characteristic of species, and for some species may allow tentative identification by the experienced worker. Routine recognition of *Bacillus thuringiensis* is largely dependent on observation of its cuboid or diamond-shaped parasporal crystals in sporangia (Figure 10h).

Nutrition and growth conditions. Despite the very wide diversity of the genus, most *Bacillus* species will grow well on routine media such as nutrient agar or trypticase soy agar, and most will grow on blood agar. However, some isolates, particularly those from nutritionally poor environments, may grow poorly if at all on these standard media and so require weaker formulations; for example, strains of *Bacillus thuringiensis* (Forsyth and Logan, 2000) isolated from Antarctic soils required *Bacillus fumarioli* agar or a half-strength formulation of this medium for reliable cultivation, and they would not grow consistently on trypticase soy agar.

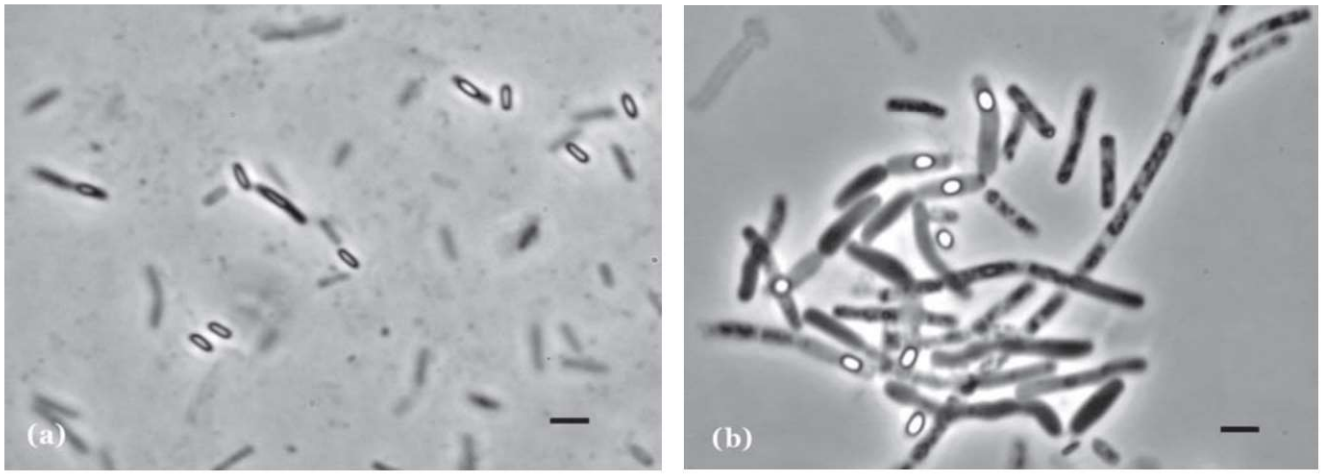

FIGURE 10. Photomicrographs of *Bacillus* species viewed by phase-contrast microscopy. Bars = 2 μm. (a) *Bacillus pumilus*: slender cells with cylindrical, subterminal spores, not swelling the sporangia; (b) *Bacillus cereus*: broad cells with ellipsoidal, paracentral and subterminal spores, not swelling the sporangia and showing some poly-β-hydroxybutyrate inclusions, which are smaller and less phase-bright than the spores; (c) *Bacillus circulans*:

(continued)

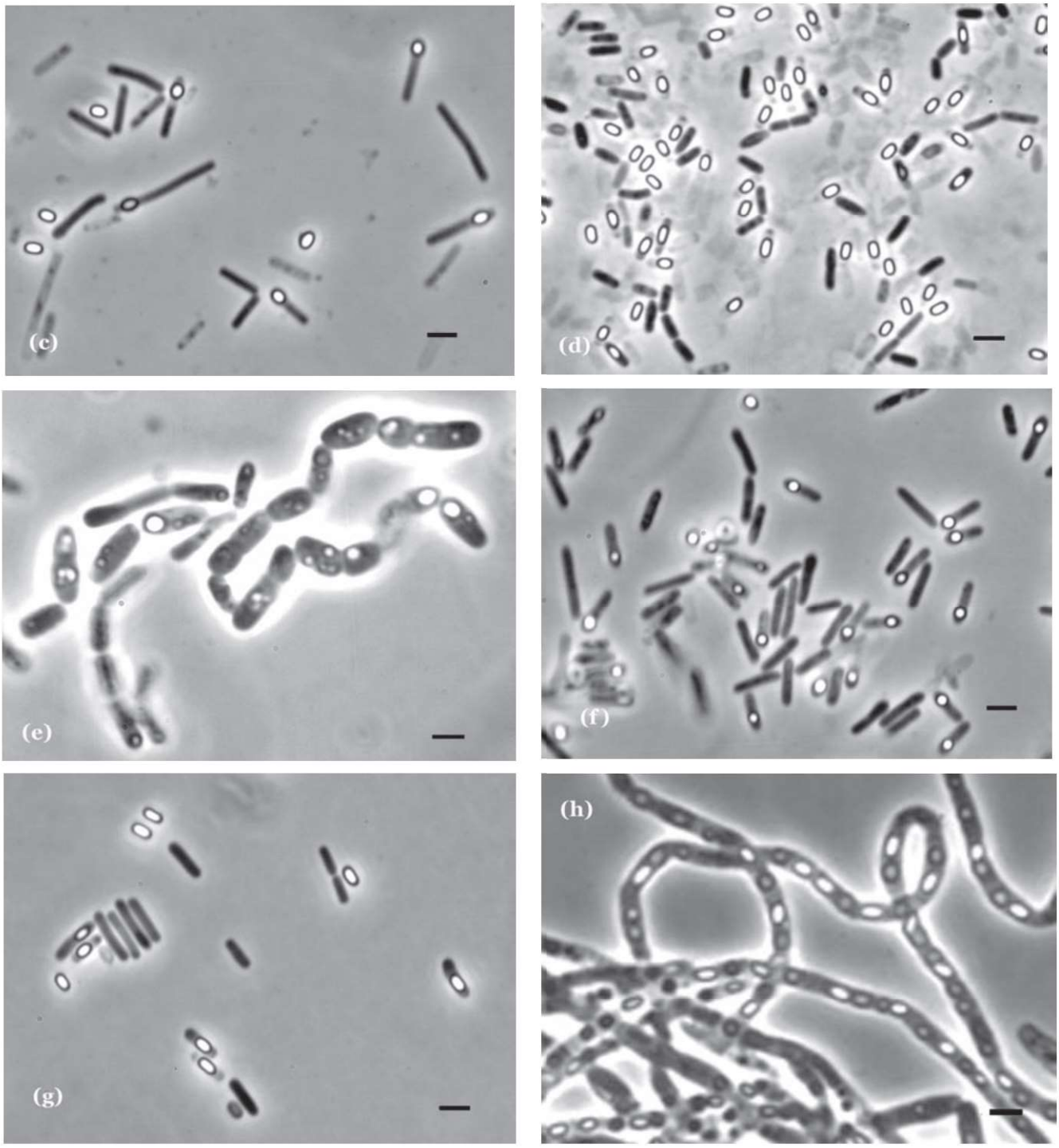

FIGURE 10. (continued) ellipsoidal, subterminal spores, swelling the sporangia; (d) *Bacillus licheniformis*: some chaining of cells evident; ellipsoidal, central and subterminal spores, not swelling the sporangia; (e) *Bacillus megaterium*: broad cells with ellipsoidal to spherical, subterminal and terminal spores, not swelling the sporangia, and showing poly-β-hydroxybutyrate inclusions, which are smaller and mostly less phase-bright than the spores; (f) *Bacillus sphaericus*: spherical, terminal spores, swelling the sporangia; (g) *Bacillus subtilis*: ellipsoidal, central, paracentral and subterminal spores, not swelling the sporangia; (h) *Bacillus thuringiensis*: broad cells with ellipsoidal, subterminal spores, not swelling the sporangia, and showing parasporal crystals of insecticidal toxin, which are less phase-bright than the spores. Photomicrographs prepared by N. A. Logan.