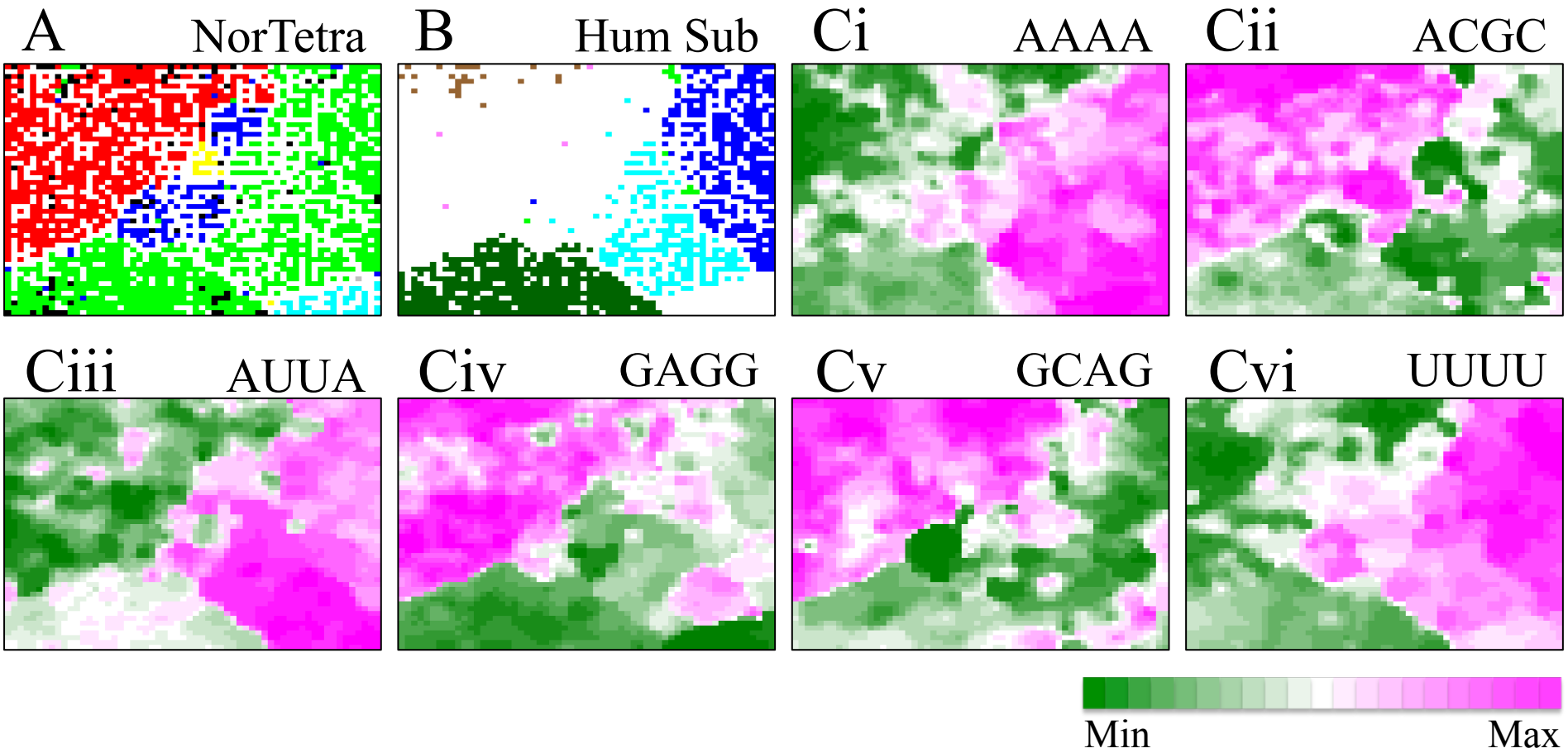Additional file 2